CHAPTER 6

Chemopreventive potential of Nyctanthes arbor-tristis and Phlogacanthus thyrsiflorus

6.1 Background

Cancer stands as one of the most aggressive and lethal diseases in the present scenario of disease burden throughout the world. Although surgery, chemotherapy and radiotherapy are commonly used therapeutic modalities for cancer, they have limited benefits to the patients due to metastasis, acquired chemoresistance and issues related to the toxicity. [287,288] Therefore, the research on anticancer drug development has been shifted towards a more realistic and holistic strategy using naturally occurring non-toxic chemical entities. The term 'chemoprevention', refers to inhibition or reversal of the process of carcinogenesis before the development of invasive cancer using nontoxic drugs derived from plants and other sources. [16,17] Studies have demonstrated that regular consumption of fruits and vegetables rich in phytochemicals reduces the risk of several degenerative diseases including cancer. [289,290] These phytochemicals derived from dietary foods and vegetables have been reported to interfere with the 'biochemical' and 'molecular' mechanisms of carcinogenesis process and thereby reduce the risks. [17] But, the desired level of efficacy in chemoprevention and anticancer potentials, with no or least side effects and low cost is yet to be achieved. Therefore, identification of novel phytochemicals based on traditional and indigenous knowledge of the medicinal plant, has gained impetus in recent past.

With this background, in this study, we have investigated and established for the first time the chemopreventive potential of the flower extract *Nyctanthes arbor-tristis* (FENA) and leaf extract of *Phlogacanthus thyrsiflorus* (LEPT) against DMBA induced and croton oil promoted skin papillomagenesis and Benzo(a)pyrene induced forestomach papillomagenesis in *Swiss albino* mice. The histopathological consequences of papillomagenesis and chemoprevention were evaluated. To examine the biochemical mechanism of observed chemopreventive potentials, we have also evaluated the modulatory influence of FENA and LEPT on the activity/level of phase I and phase II xenobiotic metabolizing enzymes, antioxidants and reactive species in the liver, extra-hepatic and papilloma bearing tissues.

6.2 Materials and methods

6.2.1 Chemicals and reagents

7,12-Dimethylbenz(a)anthracene (DMBA), croton oil, 2′,7′-Dichlorofluorescin diacetate (DCFD), Benzo(a)pyrene, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), oxidized glutathione (GSSG), pyrogallol, 2,6-dichlorophenol-indophenol (DCPIP), potassium ferricyanide, triton X-100, ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA), sodium pyruvate, thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma-Aldrich, USA. The rest of the chemicals utilized were obtained from local firms (India) and were of analytical grade.

6.2.2 Experimental design

Random-bred male *Swiss albino* mice of 6–8 weeks old were used for the study. The animals were maintained in the air-conditioned animal house facility at Jawaharlal Nehru University, New Delhi (India) and Tezpur University, Tezpur, Assam (India) with a 12 hrs light/12 hrs dark cycle and provided (unless otherwise stated) with standard food pellets and drinking water *ad libitum*. The experiment was approved by the Jawaharlal Nehru University Animal Ethics Committee (JNUAEC) and Tezpur University Animal Ethical Committee (TUAEC) [Annexure IV]. The experimental animals were acclimatized for 1 week before starting the experiments. The experimental animals were cared for according to the standard procedure as described by “Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)” and guidelines of Institutional Animal Ethical Committee (JNUAEC/TUAEC).

6.2.2.1 Experiment 1: Modulatory effect of FENA and LEPT on the enzymes of xenobiotic metabolism, antioxidant system , toxicity related parameters and detoxification of reactive species

The experimental animals of equivalent weight were assorted into following four groups [Figure 19].

Group I (n =6): The animals of this group were not treated with FENA/LEPT and served as control.

Group II (n =6): The animals of this group received 25 mg/kg body weight of FENA/LEPT in drinking water for 2 weeks.

Group III (n =6): The animals of this group received 50 mg/kg body weight of FENA/LEPT in drinking water for 2 weeks.

Group IV (n =6): The animals of this group received 100 mg/kg body weight of FENA/LEPT in drinking water for 2 weeks.

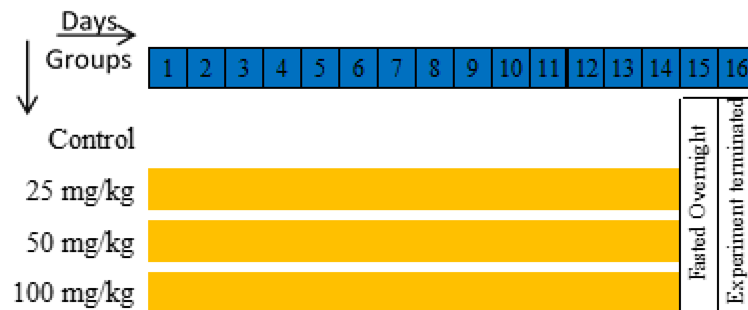


Figure 19: Timeline of treatment schedule of modulatory influence of FENA/LEPT: *Swiss albino* mice were fed with FENA/LEPT (25, 50 and 100 mg/kg body weight/day) with drinking water for a 15 days. The blue line and orange line represent days and FENA/LEPT treatment respectively.

6.2.2.2 Experiment 2: Chemopreventive potential of FENA and LEPT against skin papillomagenesis

DMBA induced and croton oil promoted skin papillomagenesis was carried out according to the method described by Nanta and Kale (2011) and Kumar *et al.*, (2013) with some modifications. [291,292] The experiment was divided into four parts comprising of eight groups and all the animals were put on normal diet and given water *ad libitum* [Figure 20]. The hairs on the dorsum (2 cm diameter) of the mice were clipped off three days before the application of the DMBA and/or acetone to achieve the resting phase of hair growth cycle.

Control groups:

Group I (n =6): The animals of this group were topically treated with 100 µl acetone on day 14th and twice a week from the day 28th to till termination of experiments (120 days). This group of animals served as negative control.

Group II (n =6): The animals of this group were treated with a single dose of DMBA (100 µg in 100 µl acetone) topically on shaved dorsal area of skin as papilloma initiator. Two weeks after the carcinogen application, 100 µl of 1% croton oil in acetone was topically applied on same area of carcinogen application as papilloma promoter twice a week until termination of the experiment. This group served as positive control.

Anti-initiation groups:

Group III (n=6): The animals of this group received FENA/LEPT 50 mg/kg body weight with drinking water starting 2 weeks before application of DMBA and discontinued just before first application of croton oil. DMBA and croton oil were applied as per group II.

Group IV (n=6): The animals of this group received FENA/LEPT 100 mg/kg body weight with drinking water starting 2 weeks before application of DMBA and discontinued just before first application of croton oil. DMBA and croton oil were applied as per group II.

Anti-promotion groups:

Group V (n=6): The animals of this group received FENA/LEPT 50 mg/kg body weight with drinking water from the day of croton oil treatment (*i.e.* 28th day) until the termination of experiment. DMBA and croton oil were applied as in positive control group.

Group VI (n=6): The animals of this group received FENA/LEPT 100 mg/kg body weight with drinking water from the day of croton oil treatment (*i.e.* 28th day) until the termination of experiment. DMBA and croton oil were applied as in positive control group.

Figure 20: Timeline of treatment schedule of chemopreventive potentials of FENA/LEPT against DMBA induced and croton oil promoted skin papillomagenesis: Male *Swiss albino* mice of 6-8 weeks were fed with FENA/LEPT (50 and 100 mg/kg body weight/day) at pre-initiation, post-initiation and pre-post initiation stages of papillomagenesis with drinking water. A single dose of DMBA in acetone was topically applied to initiate papilloma and croton oil was used twice a week till the end of experiment (120 days) for promoting the papilloma. Representations: blue line : days; orange line : FENA/LEPT treatment; purple arrow: hair shaving; black arrow: acetone application; red arrow: DMBA application and green arrow: croton oil application.

Anti-initiation/anti-promotion groups:

Group VII (n=6): The animals of this group received FENA 50 mg/kg body weight with drinking water starting 2 weeks before application of DMBA and continued till termination of experiment. DMBA and croton oil were applied as in positive control group.

Group VIII (n=6): The animals of this group received FENA 100 mg/kg body weight with drinking water starting 2 weeks before application of DMBA and continued till termination of experiment. DMBA and croton oil were applied as in positive control group.

6.2.2.3 Experiment 3: Chemopreventive potential of FENA and LEPT against forestomach papillomagenesis

Chemopreventive potentials of FENA/LEPT against Benzo(a)pyrene induced forestomach papillomagenesis was studied as described by Wattenberg *et al.*, (1980) and Azuine and Bhide (1992) with some modifications. [293,294] The experimental animals

were assorted into the following groups and all were put on normal diet and given water *ad libitum* [Figure 21].

Group I (n =8): The animals of this group received 100 µl peanut oil twice a week, for 4 weeks from the 14th day, through oral gavage route. This group of animal served as negative control.

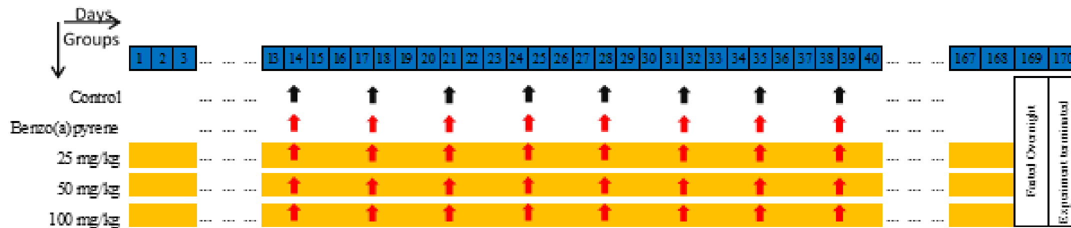


Figure 21: Timeline of treatment schedule of chemopreventive potentials of FENA/LEPT against Benzo(a)pyrene induced forestomach papillomagenesis: Male *Swiss albino* mice of 6-8 weeks were fed with FENA/LEPT (25, 50 and 100 mg/kg body weight/day) throughout the experimental period (170 days) with drinking water. A total of eight doses of B(a)P in peanut oil (twice a week) was given through oral gavage to initiate and promote forestomach papillomagenesis. Representations: blue line : days; orange line : FENA/LEPT treatment; black arrow : peanut oil treatment and red arrow: Benzo(a)pyren treatment.

Group-II (n = 8): The animals of this group received 1 mg B(a)P in peanut oil twice a week for 4 weeks from the 14th day, through oral gavage route. This group of animal served as positive control.

Group-III (n = 8): The animals of this group received LEPT 25 mg/kg body weight/mice/day with drinking from starting of the experiment to the end (170 days). This group received B(a)P as per positive control group. The dose of 25 mg/kg body weight/mice/day was not evaluated for FENA.

Group-III (n = 8): The animals of this group received FENA/LEPT 50 mg/kg body weight/mice/day with drinking from starting of the experiment to the end (170 days). This group received B(a)P as per positive control group.

Group-IV (n = 8): The animal of this group received FENA/LEPT 100 mg/kg body weight/mice/day with drinking from starting of the experiment to the end. This group received B(a)P as per positive control group.

Body weights of the animals in all groups were recorded initially, at weekly intervals (3 days interval in case of experiment 1) and at termination of the experiment. In addition, animals were monitored for toxicity, mortality, food and water consumption daily. At the end of mentioned study period, photographs of mice were taken and skin papilloma were counted. In case of forestomach papillomagenesis, papilloma were counted under magnifying glass, after dissecting out the forestomach. The parameters related to papilloma development were calculated in terms of papilloma incidence (number of mice carrying at least one papilloma), inhibition of papilloma multiplicity (percentage inhibition of papilloma as compared to positive control), papilloma yield (average number of papilloma per mouse) and papilloma burden (average number of papilloma per papilloma bearing mice) and finally expressed as percentage change to control group.

6.2.3 Histological studies

At the end of the experiment, papilloma bearing tissues *viz.* skin and forestomach were harvested and fixed in 10% formalin buffer. In case of negative control group, only normal tissue sample was taken. The fixed tissue samples were dehydrated through ascending grades of alcohol, cleared in benzene and embedded in low melting paraffin. The embedded tissue samples were sectioned in a size of five micron thickness, placed on glass slides and then deparaffinized through descending grades of alcohol. The sections made from each of the tissues were stained with haematoxylin and eosine for histological study.

6.2.4 Preparation of homogenate of hepatic tissue

At the end of experiments, animals were sacrificed by cervical dislocation and the entire liver was then perfused immediately with cold 0.9% NaCl. Thereafter the liver was carefully removed, trimmed free of extraneous tissue and rinsed in chilled 0.15 M Tris-KCl buffer (0.15 M KCl + 10 Mm Tris-HCl, pH 7.4). The liver was then blotted dry,

weighed quickly and homogenized in ice cold 0.15 M Tris-KCl buffer (pH 7.4) to yield 10% (w/v) homogenate. The tissue homogenates were centrifuged at 10,000 rpm for 20 minutes and the supernatants were collected. The resultant supernatants were transferred into pre-cooled centrifugation tubes, subjected to high speed centrifugation for 60 min and the supernatant containing cytosolic fraction was used for biochemical estimation.

6.2.5 Preparation of homogenate of extra-hepatic organs and papilloma bearing tissues

The lung, kidneys, forestomach and skin samples were carefully removed, along with the liver, trimmed free of extraneous tissue and rinsed in chilled 0.15 M Tris-KCl (pH 7.4). The skin and lung were cut into small pieces. The stomach was opened longitudinally; the forestomach was separated from the glandular stomach and cleaned of all its contents by flushing with and changing the buffer 5-6 times. The collected tissues were then blotted dry, weighed quickly and homogenized in ice cold 0.15 M Tris-KCl buffer (pH 7.4) to yield a 10% (w/v) homogenate. The homogenates were centrifuged at 15,000 × g for 30 min at 4°C and the resultant supernatants were used for biochemical estimation.

6.2.6 Biochemical estimation in hepatic, extra-hepatic and papilloma bearing tissues

An aliquot of tissue homogenates was immediately precipitated with TCA (5%). The precipitate was removed after centrifugation and the resultant supernatant used for assaying reduced glutathione (GSH), nitric oxide (NO) and reactive oxygen species (ROS) levels. The cytosolic fractions obtained after high speed centrifugation was used for assaying phase II xenobiotic metabolizing enzymes *i.e.* glutathione-S-transferase (GST), DT-diaphorase (DTD), lactate dehydrogenase (LDH) and antioxidant enzymes *i.e.* superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx). The cytosolic pellet fraction was suspended in homogenizing buffer and used for assaying lipid peroxidation (LP) and phase I xenobiotic metabolizing enzymes *i.e.* NADPH-cytochrome P450 reductase (Cyt P450 R) and NADH-cytochrome b5 reductase (Cyt b5 R). In case of papilloma bearing tissues

and extra-hepatic tissues, the cytosolic fraction obtained only after centrifugation at 15,000 × g was used for assaying GST, DTD, SOD, CAT and LDH activity.

6.2.6.1 Estimation of Cytochrome P450 reductase (EC 1.6.2.4)

The specific activity of NADPH-cytochrome P450 reductase was assessed according to the method of Omura and Takesue (1970) with some modifications, by measuring the rate of oxidation of NADPH at 340 nm. [295] The enzymatic reaction was started at 25°C by addition of 0.1 mM NADPH to the reaction mixture containing 0.3 M potassium phosphate buffer (pH 7.5), 0.15 mM potassium ferricyanide and microsomal preparation. The enzyme activity was calculated using extinction coefficient 6.22 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as that causing the oxidation of one mole of NADPH/ min.

6.2.6.2 Estimation of Cytochrome b5 reductase (EC 1.6.2.2)

NADH-cytochrome b5 reductase was assayed according to the method of Mihara and Sato (1972), by measuring the rate of reduction of potassium ferricyanide at 420 nm by NADH. [296] The enzymatic reaction was started at 25°C by addition of 0.2 mM NADH to the reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.5), 0.75 mM potassium ferricyanide and microsomal preparation. The enzyme activity was calculated using extinction coefficient 1.02 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as that causing the reduction of 1 mole ferricyanide/min.

6.2.6.3 Estimation of Glutathione S-transferase (EC 2.5.1.18)

The activity of Glutathione S-transferase (GST) was determined in the cytosolic fraction as described in section 4.2.3.6.

6.2.6.4 Estimation of DT-diaphorase (EC 1.6.99.2)

The activity of DT-diaphorase (DTD) was measured as described by Ernster *et al.*, (1962) with NADH as the electron donor and 2,6-dichlorophenol-indophenol (DCPIP) as the electron acceptor at 600 nm. [297] The reaction was started by addition of 40 µM DCPIP to the reaction mixture consisting of 50 µM Tris-KCl buffer (pH 7.4), 0.5 mM NADH, 0.24% Triton X 100 and cytosolic preparation. The activity was calculated using

extinction coefficient 21 mM^{-1} cm^{-1}. One unit of enzyme activity has been defined as the amount of enzyme required to reduce 1 μmole DCPIP/min.

6.2.6.5 Estimation of Reduced glutathione

The level of reduced glutathione (GSH) was estimated as described in section 4.2.3.7 and finally expressed as mmole of –SH content/g tissue.

6.2.6.6 Estimation of Glutathione reductase (EC 1.8.1.7)

The activity of glutathione reductase (GR) was determined by the procedure as described by Carlberg and Mannervick (1985), measuring the rate of oxidation of NADPH at 340 nm. [298] Briefly, the reaction mixture containing 0.2 mM EDTA in 0.125 M sodium phosphate buffer (pH 7.0), 1 mM oxidized glutathione (GSSG) and 0.2 mM NADPH was started by adding cytosol to the reaction mixture and the enzyme activity was measured indirectly by monitoring the oxidation of NADPH following decrease in OD/min for 3 min at 340 nm. One unit of enzyme activity has been defined as nmoles NADPH consumed/min/mg protein based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

6.2.6.7 Estimation of Glutathione peroxidase (EC 1.11.1.9)

The activity of Glutathione peroxidase (GPx) was measured by the coupled assay method as described by Paglia and Valentine (1967). [299] Briefly, the oxidantion reaction was initiated by adding 0.2 mM NADPH to the reaction mixture consisting of 1 mM EDTA in 50 mM sodium phosphate buffer (pH 7.0), 0.24 U/ml yeast glutathione reductase, 0.3 mM GSH, 0.2 mM H_2O_2 and cytosolic sample. The decrease in absorbance was monitored for 3 minute and the activity was calculated based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}. The activity has been defined as nmoles of NADPH consumed /min/mg protein.

6.2.6.8 Estimation of Catalase (EC 1.11.1.6)

The activity of Catalase (CAT) was estimated at 240 nm by monitoring the disappearance of H_2O_2 as described by Aebi (1984). [300] The mixture contained suitably diluted cytosol in phosphate buffer (50 mM, pH 7.0) and 3 mM H_2O_2. The specific activity of catalase enzyme activity has been expressed as moles of H_2O_2 reduced/min/mg protein.

6.2.6.9 Estimation of Superoxide dismutase (EC 1.15.1.1)

The activity of superoxide dismutase (SOD) was assayed as described by Marklund and Marklund (1974), which involves inhibition of pyrogallol autoxidation at pH 8.0. [301] A single unit of enzyme was defined as the quantity of SOD required to produce 50% inhibition of autooxidation.

6.2.6.10 Estimation of Lipid peroxidation

Lipid peroxidation (LP) in liver pellet fraction, and papilloma bearing tissues were estimated as described in section 4.2.3.5.

6.2.6.11 Estimation of Lactate dehydrogenase (EC 1.1.1.27)

The specific activity of lactate dehydrogenase (LDH) was assayed in the cytosolic fraction as explained in section 4.2.3.4.

6.2.6.12 Estimation of Reactive oxygen species

The intracellular reactive oxygen species (ROS) production was estimated by using 2,7-dichlorofluorescein diacetate (DCFDA) as a probe following the method of Manna *et al.* (2011). [302] Intercellular esterases enzymatically deacetylate the DCFDA into more hydrophilic nonfluorescent reduced dye dichlorofluorescin and reactive oxygen metabolites rapidly oxidize the nonfluorescent DCFH into highly fluorescent DCF. Tissue homogenates were incubated with equal volume of the assay media (20 mM Tris–HCl, 130 mM KCl, 5 mM $MgCl_2$, 20 mM NaH_2PO_4, 30 mM glucose and 5 μM DCFDA) at 37°C for 15 min. The formation of DCF was measured at the excitation wavelength of 488 nm and emission wavelength of 610 nm for 10 min by using fluorescence spectrophotometer.

6.2.6.13 Estimation of Nitric oxide

Nitric oxide (NO) levels in the cytosolic fraction was determined as described in section 4.2.3.8 and finally expressed as μM/g tissue.

6.2.6.14 Estimation of Protein

Cytosolic and microsomal protein content was determined as described in section 4.2.3.9.

6.2.6.15 Statistical analysis

All the results were presented as Mean ± sem. Statistical analysis was performed using ANOVA following Mann-Whitney *U*-test. A value of $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$ were considered to indicate a significant difference between groups.

6.2.7 GC-MS analysis of FENA and LEPT

The bioactive components present in FENA was identified at Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu (India) by gas chromatography-mass spectrometry (GC-MS) using an Elite-5MS (30 x 0.25mm x 0.25 µm df, composed of 5% Diphenyl / 95% Dimethyl poly siloxane) (PerkinElmer, USA) column following the method of Kumaravel et al. (2010). [303] A volume of 2 µl of FENA (split ratio of 10:1) was injected into GC-MS (PerkinElmer, USA) coupled to a Clarus 500 Mass Spectrometer mass detectors using Helium (99.999%) as carrier gas at a constant flow of 1ml/min. The injector temperature was set at 250°C and ion-source temperature 280°C. The oven temperature was programmed from 110°C, with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. The total run time was 20 minutes. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

The phytochemical analysis of LEPT was carried out at CARe-KERELAM Ltd., Thrissur, Kerela (India) by GC-MS using DB-5MS column (Agilent, USA). A volume of 1 µl of LEPT was injected into GCMS (Model: 7890A, Agilent, USA) coupled to a Mass Selective Detector" (MSD) (Model : 5975C, Agilent, USA) using helium as carrier gas at a constant flow of 1ml/min under under electron impact ionization (70 eV). The stepped temperature program was held at 60°C for 2 min, increased from 60°C to 250°C at 5°C per minute, and held for 5 min. The total run time was 60 minutes. The mass spectra or peaks were interpreted by computer searching in a commercial mass spectral reference library (Wiley NIST)

6.2.8 *In silico* ADME/Tox analysis

The pharmacokinetic parameters, absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) of identified phytochemicals were evaluated by *in silico* method. The canonical SMILES of the selected phytochemical (abundance greater than 5%) were generated by using online smiles translator and structure file generator tools (http://www.cactuc.nci.nih.gov/translate/ , http://www.chemicalize.org/) and thereafter subjected to ADME/Tox analysis using online software pkCSM pharmacokinetics (http://bleoberis.bioc.cam.ac.uk/pkcsm). [304]

6.3 Results

6.3.1 Modulatory influence and chemopreventive potential of FENA

6.3.1.1 General observation and weight gain profile

To determine the effect of FENA consumption, *Swiss albino* mice treated with FENA in drinking water and its effect on overall body weight gain and relative weight gain were examined at initial and end of experiment. The oral treatment of mice with FENA did not cause any apparent clinical signs of mortality, or any gross visible changes attributable to toxicity in treated mice. As shown in Table 10-12, treatment with either FENA or both the carcinogen alone significantly decreases the rate of general body weight gain as compared to negative control group. The results also depicted that, treatment of mice with FENA in pre-initiation phases significantly restored the rate of body weight gain profile in a positive correlation with relative weight gain of lung and kidneys [Table 11]. However, the increase in the relative weight gain profile of liver, lung and kidneys in post-peri-initiation phases cannot be correlated with the rate of body weight gain [Table 11]. Treatment with FENA significantly restored the decreased rate of body weight gain as compared to B(a)P treated group along with liver and forestomach [Table 12].

6.3.1.2 Tumor quantification

The chemopreventive potential of FENA was determined against topically applied DMBA induced and croton oil skin tumorigenesis. As shown in Figure 22(A), there was

evidence of decrease in the skin tumor incidence upto 67 % and inhibition of tumor multiplicity upto 30% by the FENA administration. Tumor yield and burden were decreased upto 65% and 48% respectively in 50 mg/kg FENA treated anti-promotion groups, as compared to positive control group [Figure 22(B)]. Oral feeding of FENA inhibited the forestomach papilloma multiplicity upto 46.2% and 61.5% in the 50 mg/kg and 100 mg/kg FENA treated group respectively [Figure 23(A)]. Papilloma yield and burden showed similar trend of decrease and declined to 53.8% and 38.5% in the 50 mg/kg and 100 mg/kg FENA treated group respectively in comparison to only B(a)P treated group [Figure 23(B)].

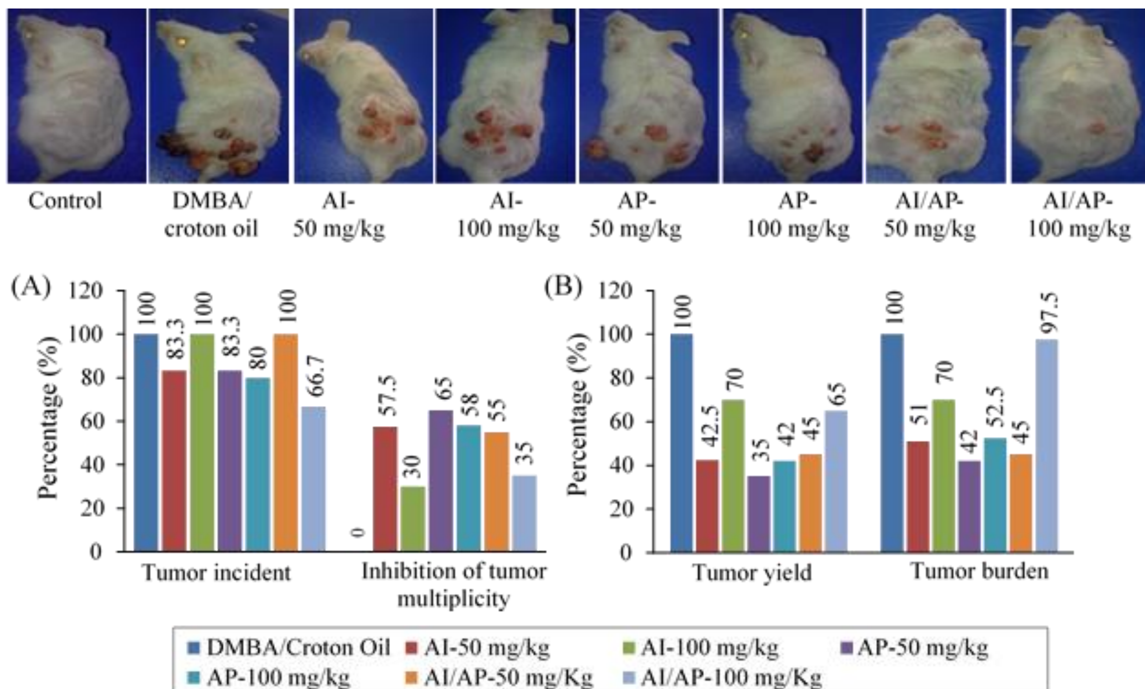


Figure 22: FENA inhibit the (A) papilloma incidence and multiplicity, (B) papilloma yield and burden against DMBA induced and croton oil promoted skin papillomagenesis: Skin papilloma were induced by DMBA and promoted by croton oil in *Swiss albino* mice. Mice were also fed with FENA in pre-initiation, post initiation and peri-initiation phase as described in 'Material and Method'. At the end of the experiment photographs of each mouse were taken and papilloma were counted. The upper panel represent the representative photographs of mice from each group**.**

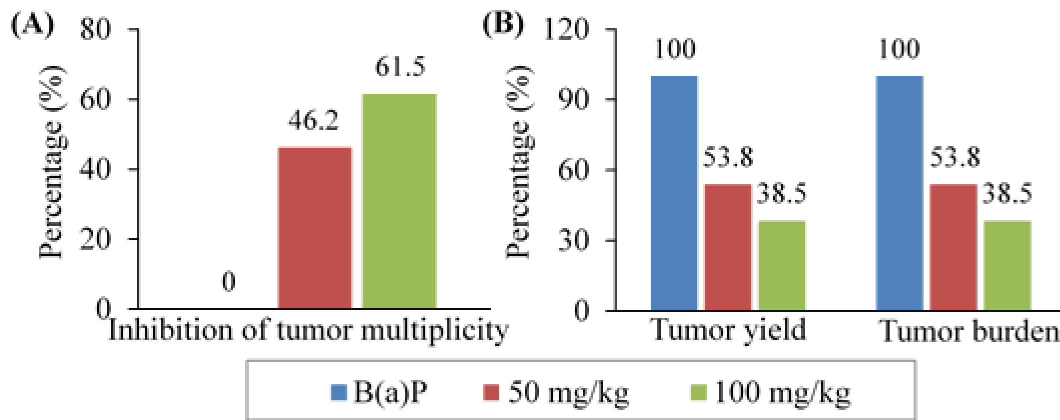


Figure 23: FENA inhibit the (A) papilloma multiplicity, (B) papilloma yield and burden against B(a)P induced forestomach papillomagenesis: Forestomach papillomas were induced by Benzo(a)pyrene in *Swiss albino* mice and mice were fed with FENA as described in 'Material and Method'. At the end of the experiment, mice were sacrificed, forestomach were collected and papillomas were counted under magnifying glass.

6.3.1.3 Histopathological examination of tumor

The histological basis of tumorigenesis and chemoprevention was examined in the skin and forestomach tissue of normal and tumor bearing mice. As shown in Figure 24, the cellular architecture of skin of control group appeared with distinct epidermal and dermal layers. In DMBA/croton oil treated group, skin exhibited tumor related characteristics like papillomatosis, acanthosis, hyperkeratosis, dermal infiltration etc. Treatment of tumor induced mice with FENA at different phase of tumorigenesis progressively altered the tumor related characteristics. Histopathological sections from the forestomach of untreated mice exhibited normal histology with well differentiated stratified squamous epithelium on the top of muscular mucosae, submucosa and mascularpropria. The histology of forestomach from the animals treated with B(a)P exhibited loss of differentiation in squamous epithelial cells along with papillomatosis, acanthosis, hyperkeratosis, and dermal infiltration. With respect to B(a)P treated group, the sections from the FENA treated group showed varying degree of improvement in the histology of mice [Figure 25].

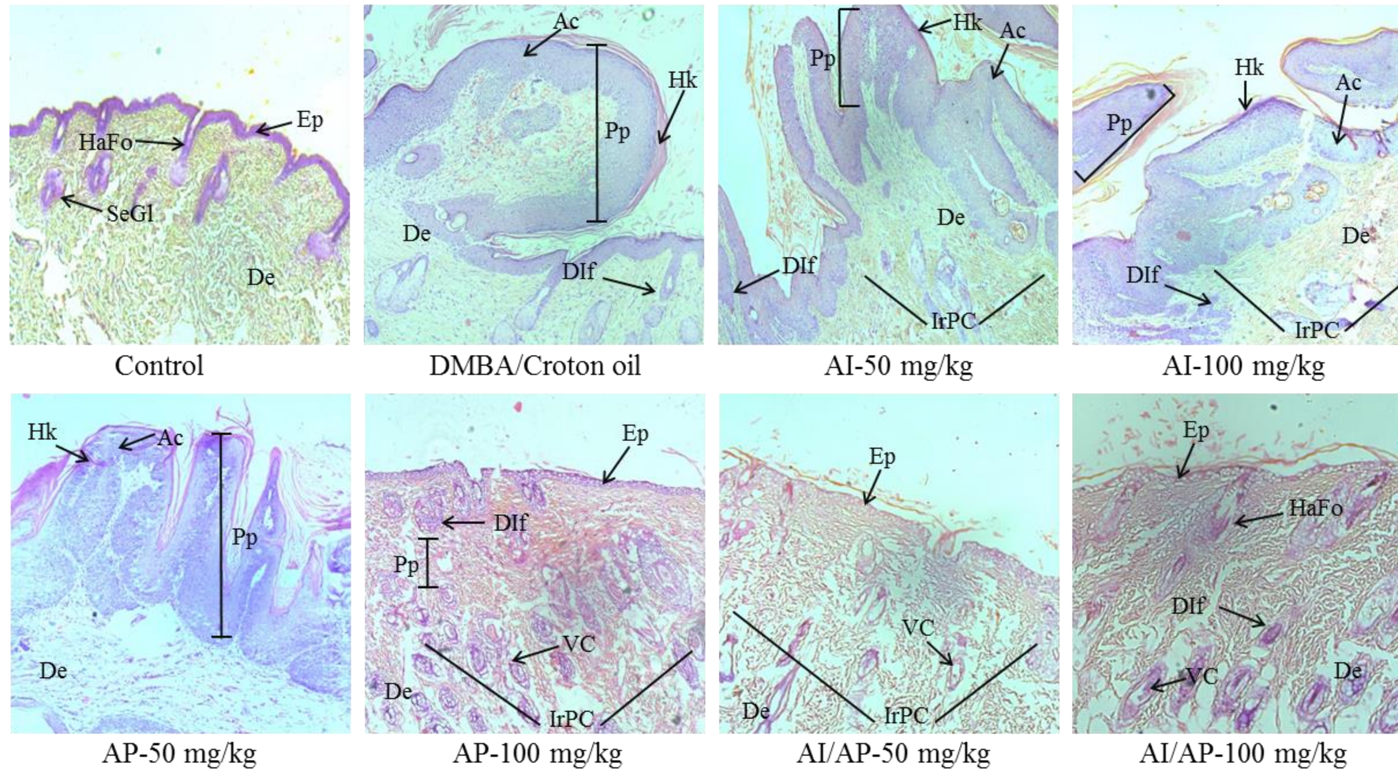


Figure 24: Hematoxylin and eosin stained sections of skin tissues of normal and papilloma bearing mice: Papillomas were chemically induced as described in 'Material and Method'. At the end of the experiment, mice were sacrificed, tissues harvested and processed for microscopy observation as described in 'Material and Method'. The photographs shown (Magnification: 100×) are the representative from each group**.** Abbreviations: Ep: epidermis; HaFo: Hair follicle; SeGl: Sebacious glang; De: Dermis; Ac: Acanthosis; Hk: Hyperkeratosis; Pp: Papillomatosis; Dif: Dermal infiltrations; IrPC: Irregular proliferation of cells and VC: Vascular congestion.

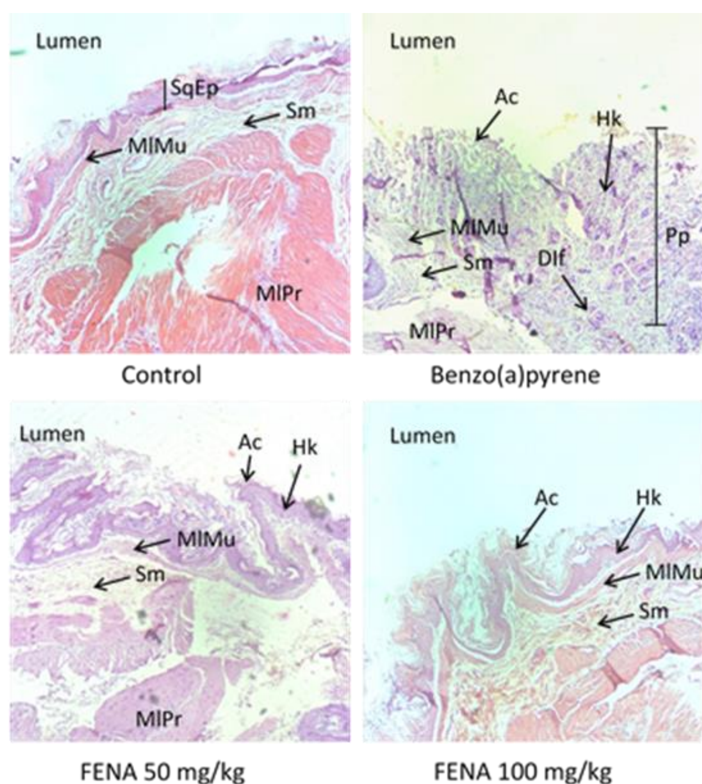


Figure 25: Hematoxylin and eosin stained sections of forestomach tissues of normal and papilloma bearing mice: Papilloma were chemically induced as described in 'Material and Method'. At the end of the experiment, mice were sacrificed, tissues harvested and processed for microscopy observation as described in 'Material and Method'. The photographs shown (Magnification: 100×) are the representative from each group**.** Abbreviations: Ac: Acanthosis; Hk: Hyperkeratosis; Pp: Papillomatosis; Dif: Dermal infiltrations; VC: Vascular congestion; SqEp: Stratified squamous epithelium; MlMu: Mascular mucosae; Sm: Submucosa; MlPr: Mascular propria.

6.3.1.4 Biochemical estimation in hepatic tissue

As the liver is the master gland responsible for conversion of pre-carcinogen into carcinogen, detoxification of xenobiotic/carcinogens, we examined the status of enzymes involved in xenobiotic metabolism, antioxidant, toxicity related parameters, in order to understand the biochemical mechanism of chemoprevention. The specific activities of phase I xenobiotic metabolism enzyme *i.e.* Cyt P450R and Cytb5 R significantly decreased in the FENA treated groups as compared to both only carcinogen

treated group [Table 14,15]. In contrast to the phase I enzymes, the activity of phase II xenobiotic metabolizing enzymes *i.e.* GST and DTD were significantly induced in comparison to control group [Table 13]. The induced activity of GST and DTD were significant in comparison to DMBA/croton oil and B(a)P treated group respectively [Table 14,15]. FENA was found to be effective in significantly elevating the hepatic non enzymatic antioxidant, GSH [Table 13] and the elevation was observed to be significant against DMBA/croton oil treated group [Table 14]. The two major antioxidant enzymes *i.e.* SOD and catalase were significantly increased upon FENA treatment in comparison to control group [Table 13] as well as carcinogen treated groups [Table 14,15]. The activity of hepatic GR and GPx were also observed to be significantly increased in comparison to control group [Table 13] and B(a)P treated group [Table 15]. Lipid peroxidation in the membranous fraction of liver was found to be significantly increased in both the carcinogen treated group [Table 14,15] and treatment with FENA significantly augmented the level of MDA as compared to only B(a)P treated group [Table 15]. However, the level of MDA formation in FENA treated groups was observed to be in a comparable range with negative control group [Table 13-15]. The specific activity of LDH and level of NO and ROS were found to be significantly decreased in the FENA treated groups as compared to carcinogen treated groups [Table 14,15].

6.3.1.5 Biochemical estimation in papilloma bearing tissues

The activities of GST, SOD, catalase and level of GSH were significantly augmented following FENA treatment in the papilloma bearing skin samples as compared to DMBA/croton oil treated group [Table 16]. FENA treatment also significantly restored the activity of GST, DTD and level of GSH in forestomach as compared to the B(a)P treated group [Table 16]. Treatment with FENA significantly augmented the level of LP and ROS in both skin and forestomach papilloma bearing tissues in comparison to both the carcinogen treated groups [Table 16,17]. A decrease in the activity of LDH and NO levels were also observed following FENA treatment as compared to positive control groups [Table 16,17].

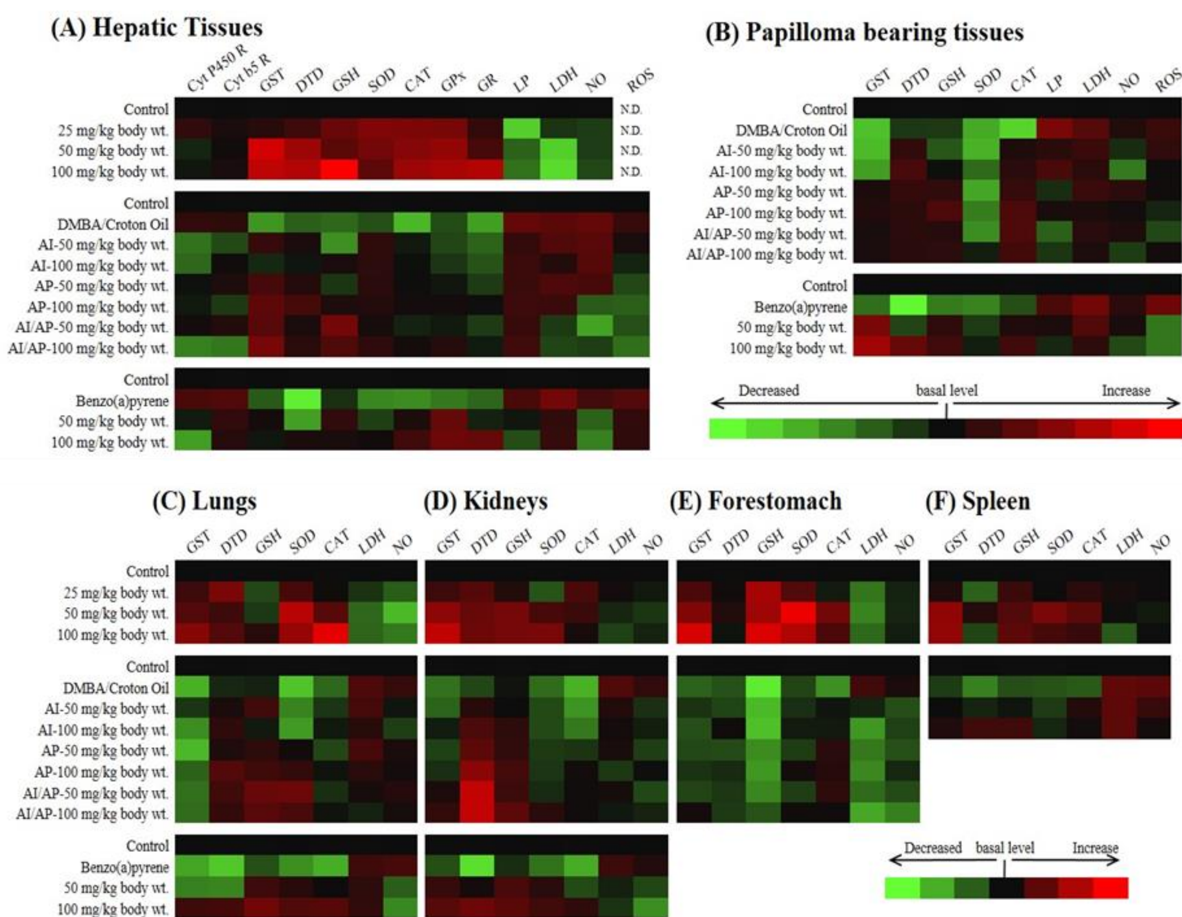


Figure 26: Heat map showing the modulatory influence of FENA : The activities/levels of enzymes involved in xenobiotic metabolism, antioxidants, toxicity related parameters and reactive species were measured in the hepatic, extra-hepatic as well as papilloma bearing tissues of Swiss albino mice treated with FENA or carcinogen or FENA + carcinogen. Values are expressed as mean (N = 4 to 6) fold change in the activity/level in comparison to control.

6.3.1.6 Biochemical estimation in extra-hepatic tissues

FENA was found to be effective in elevating activities/level of GST, DTD, SOD and CAT in lung; GST, DTD and SOD in kidneys; GST, GSH, SOD; and CAT in forestomach and GST, SOD and CAT in spleen [Table 18,19]. There was evidence of significant decline in the level of NO in lung as compared to control [Table 18]. Treatment with FENA significantly restored the activities/levels of DTD, GSH, SOD in lung; GST, DTD, GSH and CAT in kidney; and GSH and CAT in forestomach as

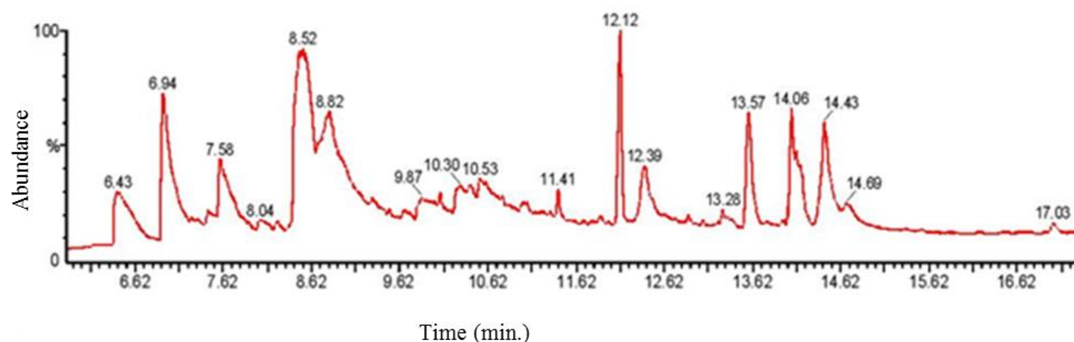
compared to the DMBA/croton oil treated group [Table 20-22]. Oral feeding of FENA significantly augmented the activity of LDH and level of NO in lung, kidneys and forestomach as compared to only DMBA/croton oil treated group [Table 20-22]. There was evidence of significant restoration in the activities/levels of DTD, GSH, SOD and CAT in lung; GST, DTD, GSH, SOD and CAT in kidneys; and DTD, GSH and CAT in spleen as compared to only B(a)P treated positive control group [Table 23,24]. FENA was also found to be effective in significant restoration of NO level in lung, kidneys and spleen; and activity of LDH in spleen as compared to only B(a)P treated group [Table 23,24].

6.3.2 Phytochemical characterization of FENA by GC MS

The GC-MS analysis indicated the presence of 14 Nos. of phytochemicals in FENA. The prevailing compounds were Dodecanoic acid, 3-hydroxy- (26.41%), 2,5-Methano-furo[3,2-b]pyran, hexahydro (13.56%), Oleic acid acid (8.68%), 9-Oxa-bicyclo[3.3.1]nonane-1,4-diol (8.59%) and 9,12-Octadecadienoic acid, ethyl ester (8.54%) that constitute about 66% of total phytoconstituents present in FENA [Figure 27]. [Appendix V]

6.3.3 Pharmacokinetic profile of phytochemicals present in FENA

The pharmacokinetic profiling was analyzed by studying absorption, distribution, metabolism, excretion and toxicological parameters using online ADME/Tox software. The results of the *in silico* analysis indicated that most of the phytochemicals resent in FENA have high intestinal absorption capability (91-100%) and permeability to cells. The phytochemical hexadecanoic acid, phytol, 9,12-Octadecadienoic acid, ethyl ester can also cross blood brain barrier. None of them were found to be genotoxic, hepatotoxic. The maximum tolerant dose of human for these phytochemicals varies from 0.73-1.64 mg/kg body weight/day. The oral chronic (LD50) and acute rat toxicity were found to be considerable moderate range 1.79-2.35 mg/kg/day and 1.08-12.578 mol/kg [Table 25].



No	RT	Name of compound	Molecular formulae	MW	Peak area (%)
1	6.43	9-Oxa-bicyclo[3.3.1]nonane-1,4-diol	$C_8H_{14}O_3$	158	8.59
2	6.94	2,5-Methano-2H-furo[3,2-b]pyran, hexahydro	$C_8H_{12}O_2$	140	13.56
3	7.58	e-10-Pentadecenol	$C_{15}H_{30}O$	226	7.55
4	8.52	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	216	26.41
5	10.53	a-D-Glucopyranose, 4-O-a-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	4.81
6	12.12	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	6.45
7	12.39	n-Heaxdecanoic acid	$C_{18}H_{32}O_2$	256	5.44
8	13.57	Phytol	$C_{20}H_{40}O$	296	5.93
9	14.06	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	8.54
10	14.43	Oleic acid	$C_{18}H_{34}O_2$	282	8.68

Figure 27: Total ion Chromatogram (TIC) of FENA: GC-MS analysis was done using Elite-5MS (30 x 0.25mm x 0.25 m df, composed of 5% Diphenyl / 95% Dimethyl poly siloxane) column in a GC clarus 500 Perkin Elmer system. Only the major phytochemicals present in FENA are shown here.

Table 10: Effects of FENA on general body weight gain profile and relative organ weight in untreated *Swiss albino* mice

Groups	Body Weight (gms)		Relative weight of organs				
	Initial	Final	Liver	Lungs	Kidneys	Forestomach	Spleen
Control	22.833 ± 0.833 (1.000)	30.500 ± 0.885 (1.336)^{a*}	6.108 ± 0.682 (1.000)	0.574 ± 0.058 (1.000)	1.410 ± 0.059 (1.000)	0.217 ± 0.020 (1.000)	22.833 ± 0.833 (1.000)
25 mg/kg body wt./mice/day	27.833 ± 0.307 (1.000)	34.667 ± 0.882 (1.246)^{a*}	5.738 ± 0.388 (0.939)	0.538 ± 0.050 (0.937)	1.375 ± 0.060 (0.975)	0.206 ± 0.020 (0.951)	27.833 ± 0.307 (1.000)
50 mg/kg body wt./mice/day	32.000 ± 0.447 (1.000)	39.333 ± 0.715 (1.229)^{a*c}	5.330 ± 0.404 (0.873)	0.572 ± 0.018 (0.996)	1.309 ± 0.081 (0.929)	0.187 ± 0.014 (0.864)	32.000 ± 0.447 (1.000)
100 mg/kg body wt./mice/day	32.000 ± 0.683 (1.000)	36.167 ± 1.108 (1.130)^{b*b}	5.500 ± 0.253 (0.900)	0.514 ± 0.048 (0.896)	1.485 ± 0.030 (1.053)	0.196 ± 0.025 (0.904)	32.000 ± 0.683 (1.000)

Values are expressed as mean ± SEM of 6 animals. Values in parentheses represent relative change in comparison to control mice. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. '*' mark indicate p value in comparison to initial weight.

Table 11: Effects of FENA on general body weight gain profile and relative organ weight in DMBA and croton oil treated *Swiss albino* mice

Groups	Body Weight (gms)		Relative weight of organs			
	Initial	Final	Liver	Lungs	Kidneys	Forestomach
Control	27.667 ± 2.963 (1.000)	39.667 ± 1.667 (1.434)^{b*}	6.646 ± 0.218 (1.000)	0.592 ± 0.016 (1.000)	1.568 ± 0.032 (1.000)	0.196 ± 0.044 (1.000)
DMBA/Croton Oil	38.167 ± 0.833 (1.000)	47.333 ± 0.333 (1.240)^{a*}	5.352 ± 0.262 (0.805)^{b}	0.524 ± 0.021 (0.886)^{c}	1.345 ± 0.026 (0.858)^{b}	0.182 ± 0.012 (0.926)
AI 50 mg/kg body wt./mice/day	32.833 ± 0.477 (1.000)	46.000 ± 0.577 (1.401)^{a*d}	5.772 ± 0.370 (0.868)	0.573 ± 0.023 (0.969)	1.569 ± 0.071 (1.001)^{f}	0.176 ± 0.012 (0.898)
AI 100 mg/kg body wt./mice/day	31.333 ± 0.558 (1.000)	41.833 ± 0.307 (1.335)^{a*d}	6.311 ± 0.381 (0.949)	0.718 ± 0.067 (1.213)^{f}	1.691 ± 0.062 (1.079)^{d}	0.173 ± 0.017 (0.880)
AP 50 mg/kg body wt./mice/day	35.167 ± 0.477 (1.000)	44.833 ± 0.654 (1.275)^{a*}	5.976 ± 0.389 (0.899)	0.548 ± 0.035 (0.926)	1.460 ± 0.047 (0.931)	0.231 ± 0.022 (1.177)
AP 100 mg/kg body wt./mice/day	35.000 ± 0.365 (1.000)	44.400 ± 0.600 (1.269)^{a*}	6.309 ± 0.358 (0.949)	0.534 ± 0.041 (0.903)	1.468 ± 0.011 (0.937)^{e}	0.219 ± 0.016 (1.117)
AI/AP 50 mg/kg body wt./mice/day	35.500 ± 1.147 (1.000)	44.000 ± 1.155 (1.239)^{a*}	6.584 ± 0.227 (0.991)^{e}	0.642 ± 0.016 (1.085)^{e}	1.830 ± 0.049 (1.167)^{bd}	0.187 ± 0.013 (0.954)
AI/AP 50 mg/kg body wt./mice/day	36.500 ± 0.764 (1.000)	43.500 ± 1.232 (1.192)^{a*c}	6.009 ± 0.427 (0.904)	0.601 ± 0.041 (1.016)	1.606 ± 0.042 (1.024)^{d}	0.184 ± 0.024 (0.939)

Values are expressed as mean ± SEM of 4-6 animals. Values in parentheses represent relative change in comparison to control mice. $^{a}p\leq0.001$, $^{b}p\leq0.01$ and $^{c}p\leq0.05$ represent significant changes against control group. $^{d}p\leq0.001$, $^{e}p\leq0.01$ and $^{f}p\leq0.05$ represent significant changes against only DMBA/Croton oil treated group. '*'mark indicate p value in comparison to initial weight.

Table 12: Effects of FENA on general body weight gain profile and relative organ weight in Benzo(a)pyrene treated *Swiss albino* mice

Groups	Body Weight (gms)		Relative weight of organs				
	Initial	Final	Liver	Lungs	Kidneys	Forestomach	Spleen
Control	29.800 ± 0.735 (1.000)	37.840 ± 3.090 (1.270)[c*]	6.395 ± 0.522 (1.000)	0.978 ± 0.080 (1.000)	1.850 ± 0.151 (1.000)	0.484 ± 0.039 (1.000)	0.647 ± 0.053 (1.000)
Benzo(a)pyrene	27.914 ± 1.425 (1.000)	21.140 ± 0.940 (0.757)[b*c]	9.271 ± 1.548 (1.450)[c]	1.170 ± 0.220 (1.197)	2.064 ± 0.282 (1.116)	0.643 ± 0.045 (1.330)[c]	0.606 ± 0.101 (0.936)
50 mg/kg body wt./mcie/day	29.986 ± 1.857 (1.000)	32.180 ± 4.400 (1.073)[f]	7.841 ± 1.335 (1.226)	0.914 ± 0.050 (0.935)	1.716 ± 0.232 (0.928)	0.453 ± 0.119 (0.937)	0.606 ± 0.024 (0.936)
100 mg/kg body wt./mcie/day	33.686 ± 2.233 (1.000)	30.405 ± 3.595 (0.903)[c]	7.751 ± 0.269 (1.212)	0.920 ± 0.060 (0.941)	1.694 ± 0.198 (0.916)	0.471 ± 0.043 (0.975)	0.732 ± 0.052 (1.130)

Values are expressed as mean ± SEM of 4-6 animals. Values in parentheses represent relative change in comparison to control mice. [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only Benzo(a)pyrene treated group. '*' mark indicate p value in comparison to initial weight.

Table 13: Modulatory influences of FENA on the activity/level of xenobiotic metabolizing enzymes, antioxidants and toxicity related parameters in hepatic tissues of *Swiss albino* mice

Groups	Cyt P_{450} R α	Cyt b_5 R β	GST γ	DTD δ	GSH ε	SOD η
Control	0.257 ± 0.031 (1.000)	2.680 ± 0.053 (1.000)	5.182 ± 0.449 (1.000)	0.038 ± 0.003 (1.000)	3.659 ± 0.234 (1.000)	23.684 ± 0.726 (1.000)
25 mg/kg	0.288 ± 0.102 (1.121)	2.801 ± 0.073 (1.045)	5.648 ± 0.440 (1.090)	0.045 ± 0.004 (1.167)	4.784 ± 0.314 (1.307)[c]	32.858 ± 1.135 (1.387)[a]
50 mg/kg	0.249 ± 0.044 (0.971)	2.725 ± 0.280 (1.017)	8.639 ± 0.706 (1.667)[b]	0.056 ± 0.003 (1.478)[b]	4.605 ± 0.199 (1.258)[c]	31.837 ± 1.068 (1.344)[b]
100 mg/kg	0.254 ± 0.095 (0.989)	2.788 ± 0.443 (1.040)	8.262 ± 0.540 (1.594)[b]	0.059 ± 0.005 (1.556)[b]	6.610 ± 0.391 (1.806)[b]	30.067 ± 1.985 (1.270)[c]

Groups	CAT κ	GPx π	GR π	LP σ	LDH φ	NO ψ
Control	27.192 ± 1.319 (1.000)	22.476 ± 1.440 (1.000)	22.733 ± 2.794 (1.000)	0.714 ± 0.055 (1.000)	1.120 ± 0.040 (1.000)	0.910 ± 0.067 (1.000)
25 mg/kg	37.455 ± 3.261 (1.377)[c]	30.394 ± 2.305 (1.352)[c]	25.756 ± 2.766 (1.133)	0.543 ± 0.048 (0.761)[c]	1.066 ± 0.060 (0.950)	0.858 ± 0.038 (0.943)
50 mg/kg	38.144 ± 3.711 (1.403)[b]	32.509 ± 3.920 (1.446)[c]	27.207 ± 2.528 (1.197)	0.639 ± 0.036 (0.895)	0.852 ± 0.051 (0.760)[b]	0.858 ± 0.051 (0.943)
100 mg/kg	40.292 ± 3.654 (1.482)[c]	34.244 ± 3.620 (1.524)[c]	35.460 ± 2.247 (1.560)[c]	0.625 ± 0.032 (0.875)	0.833 ± 0.055 (0.744)[b]	0.844 ± 0.026 (0.928)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. α µmole of NADPH oxidized/min/mg protein, β µmole of NADH oxidized/min/mg protein, γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, π nmole of NADPH consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 14: Modulatory influences of FENA in hepatic tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	Cyt P_{450} R α	Cyt b_5 R β	GST γ	DTD δ	GSH E	SOD η	CAT κ
Control	0.283 ± 0.034 (1.000)	2.857 ± 0.031 (1.000)	4.304 ± 0.206 (1.000)	0.039 ± 0.002 (1.000)	3.422 ± 0.425 (1.000)	21.508 ± 0.703 (1.000)	22.827 ± 0.778 (1.000)
DMBA/Croton Oil	0.317 ± 0.049 (1.121)	3.170 ± 0.235 (1.110)	3.578 ± 0.303 (0.831)	0.035 ± 0.002 (0.897)	3.040 ± 0.081 (0.888)	19.726 ± 0.992 (0.917)	18.036 ± 0.503 (0.790)^{b}
AI 50 mg/Kg	0.248 ± 0.034 (0.877)	2.647 ± 0.134 (0.926)	4.888 ± 0.611 (1.136)	0.041 ± 0.003 (1.045)	2.867 ± 0.261 (0.838)	24.011 ± 3.010 (1.116)	22.514 ± 1.126 (0.986)f
AI 100 mg/Kg	0.251 ± 0.028 (0.888)	2.897 ± 0.313 (1.014)	4.163 ± 0.524 (0.967)	0.039 ± 0.001 (0.991)	3.475 ± 0.293 (1.050)	23.766 ± 2.316 (1.105)	22.762 ± 0.521 (0.997)^{d}
AP 50 mg/Kg	0.281 ± 0.020 (0.995)	3.012 ± 0.157 (1.054)	5.114 ± 0.345 (1.188)^{f}	0.042 ± 0.009 (1.076)	3.250 ± 0.255 (0.950)	23.717 ± 0.134 (1.103)^{cf}	22.981 ± 1.468 (1.007)^{f}
AP 100 mg/Kg	0.278 ± 0.011 (0.984)	2.703 ± 0.126 (0.946)	5.480 ± 0.342 (1.273)^{ce}	0.046 ± 0.006 (1.190)	3.619 ± 0.339 (1.057)	23.099 ± 1.027 (1.074)	23.432 ± 1.361 (1.026)^{f}
AI/AP-50 mg/Kg	0.291 ± 0.002 (1.028)	3.062 ± 0.113 (1.072)	5.401 ± 1.650 (1.255)	0.041 ± 0.001 (1.046)	4.595 ± 0.231 (1.343)^{e}	22.177 ± 0.040 (1.031)	22.255 ± 1.900 (0.975)
AI/AP-100 mg/Kg	0.242 ± 0.023 (0.856)^{f}	2.482 ± 0.195 (0.869)^{f}	5.804 ± 0.890 (1.349)^{f}	0.043 ± 0.003 (1.097)	4.197 ± 0.414 (1.226)^{f}	24.006 ± 3.388 (1.116)	23.442 ± 1.135 (1.027)^{e}

Table cont...

Cont.…

Groups	GPx π	GR π	LP σ	LDH φ	NO ψ	ROS ω
Control	25.612 ± 2.501 (1.000)	25.156 ± 0.282 (1.000)	0.666 ± 0.044 (1.000)	1.349 ± 0.124 (1.000)	1.200 ± 0.034 (1.000)	1.000 ± 0.023 (1.000)
DMBA/Croton Oil	23.631 ± 1.278 (0.8923)	20.621 ± 0.367 (0.820)[a]	0.862 ± 0.060 (1.293)[c]	1.699 ± 0.133 (1.259)	1.549 ± 0.056 (1.290)[b]	1.141 ± 0.082 (1.235)
AI 50 mg/Kg	23.810 ± 0.786 (0.930)	22.486 ± 2.244 (0.894)	0.751 ± 0.065 (1.126)	1.661 ± 0.126 (1.231)	1.505 ± 0.115 (1.254)[c]	1.041 ± 0.030 (1.041)
AI 100 mg/Kg	24.355 ± 1.020 (0.951)	23.034 ± 1.103 (0.916)	0.767 ± 0.024 (1.151)	1.456 ± 0.145 (1.079)	1.493 ± 0.107 (1.244)[c]	0.974 ± 0.027 (0.974)
AP 50 mg/Kg	25.307 ± 1.204 (0.988)	23.748 ± 1.738 (0.944)	0.765 ± 0.064 (1.148)	1.640 ± 0.113 (1.216)	1.436 ± 0.099 (1.196)	0.927 ± 0.038 (0.927)[f]
AP 100 mg/Kg	26.354 ± 0.973 (1.029)	25.118 ± 1.746 (0.998)	0.775 ± 0.037 (1.163)	1.560 ± 0.141 (1.156)	1.085 ± 0.057 (0.904)[d]	0.899 ± 0.027 (0.899)[cf]
AI/AP- 50 mg/Kg	25.208 ± 1.964 (0.984)	23.724 ± 1.037 (0.943)	0.758 ± 0.061 (1.138)	1.276 ± 0.091 (0.945)[f]	0.979 ± 0.021 (0.815)[bd]	0.918 ± 0.015 (0.918)[cf]
AI/AP- 100 mg/Kg	27.785 ± 2.050 (1.085)	24.740 ± 1.881 (0.983)	0.778 ± 0.041 (1.1681)	1.257 ± 0.056 (0.931)[f]	1.128 ± 0.052 (0.939)[d]	0.879 ± 0.017 (0.879)[cf]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. $^{d}p \leq 0.001$, $^{e}p \leq 0.01$ and $^{f}p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. α µmole of NADPH oxidized/min/mg protein, β µmole of NADH oxidized/min/mg protein, γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, π nmole of NADPH consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 15: Modulatory influences of FENA in hepatic tissue against B(a)P induced forestomach papillomagenesis

Groups	Cyt P_{450} R α	Cyt b_5 R β	GST γ	DTD δ	GSH Ε	SOD η	CAT κ
Control	0.285 ± 0.015 (1.000)	0.791 ± 0.039 (1.000)	5.271 ± 0.362 (1.000)	0.045 ± 0.005 (1.000)	3.740 ± 0.155 (1.000)	21.565 ± 2.274 (1.000)	26.045 ± 0.845 (1.000)
Benzo(a)pyrene	0.343 ± 0.011 (1.205)[c]	0.973 ± 0.050 (1.231)[c]	4.774 ± 0.173 (0.906)	0.033 ± 0.002 (0.719)[c]	3.578 ± 0.195 (0.957)	18.309 ± 0.650 (0.849)	21.957 ± 1.214 (0.843)
50 mg/kg	0.280 ± 0.019 (0.983)[f]	0.887 ± 0.071 (1.122)	5.426 ± 0.131 (1.030)	0.037 ± 0.001 (0.818)	4.202 ± 0.252 (1.124)	20.247 ± 0.365 (0.939)	27.501 ± 1.461 (1.056)bf
100 mg/kg	0.234 ± 0.008 (0.820)[ce]	0.858 ± 0.031 (1.085)	5.215 ± 0.090 (0.990)	0.048 ± 0.001 (1.051)[e]	3.897 ± 0.463 (1.042)	21.994 ± 1.064 (1.020)[f]	30.558 ± 2.562 (1.173)[cf]

Groups	GPx π	GR π	LP σ	LDH Φ	NO ψ	ROS ω
Control	28.151 ± 2.033 (1.000)	23.891 ± 2.335 (1.000)	0.878 ± 0.022 (1.000)	1.211 ± 0.022 (1.000)	1.037 ± 0.086 (1.000)	1.000 ± 0.017 (1.000)
Benzo(a)pyrene	24.389 ± 1.605 (0.866)	21.238 ± 1.130 (0.889)	1.055 ± 0.050 (1.202)	1.621 ± 0.027 (1.339)[a]	1.226 ± 0.076 (1.182)	1.235 ± 0.076 (1.235)[c]
50 mg/kg	36.481 ± 0.264 (1.296)[ce]	23.284 ± 0.718 (0.975)	0.868 ± 0.057 (0.989)	1.359 ± 0.087 (1.122)[f]	0.928 ± 0.050 (0.895)[f]	1.124 ± 0.087 (1.124)
100 mg/kg	37.329 ± 1.847 (1.326)[ce]	30.962 ± 0.694 (1.296)[ce]	0.808 ± 0.055 (0.921)[f]	1.374 ± 0.068 (1.134)[f]	0.888 ± 0.069 (0.857)[f]	1.115 ± 0.097 (1.115)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. α µmole of NADPH oxidized/min/mg protein, β µmole of NADH oxidized/min/mg protein, γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, π nmole of NADPH consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 16: Modulatory influences of FENA in papilloma bearing tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ
Control	0.858 ± 0.014 (1.000)	0.022 ± 0.002 (1.000)	1.804 ± 0.143 (1.000)	6.577 ± 0.481 (1.000)	16.341 ± 2.072 (1.000)
DMBA/Croton Oil	0.668 ± 0.035 (0.778)[b]	0.021 ± 0.001 (0.947)	1.706 ± 0.093 (0.946)	5.304 ± 0.103 (0.806)[c]	12.308 ± 0.982 (0.753)[c]
AI 50 mg/Kg	0.673 ± 0.039 (0.784)[b]	0.025 ± 0.002 (1.122)	1.639 ± 0.047 (0.909)	5.238 ± 0.296 (0.796)[c]	17.401 ± 1.350 (1.065)[f]
AI 100 mg/Kg	0.705 ± 0.098 (0.821)	0.027 ± 0.003 (1.198)	1.799 ± 0.076 (0.998)	5.819 ± 0.138 (0.885)	17.920 ± 1.692 (1.097)[f]
AP 50 mg/Kg	0.902 ± 0.042 (1.051)[e]	0.025 ± 0.002 (1.132)	2.024 ± 0.156 (1.122)	5.330 ± 0.391 (0.810)	18.537 ± 0.581 (1.134)[e]
AP 100 mg/Kg	0.917 ± 0.086 (1.068)	0.025 ± 0.001 (1.102)	2.201 ± 0.086 (1.221)[e]	5.664 ± 0.383 (0.861)	19.949 ± 2.045 (1.221)[f]
AI/AP-50 mg/Kg	0.893 ± 0.029 (1.041)[e]	0.025 ± 0.001 (1.102)	1.955 ± 0.088 (1.084)	5.528 ± 0.417 (0.841)	19.909 ± 1.155 (1.218)[e]
AI/AP-100 mg/Kg	0.895 ± 0.035 (1.042)[e]	0.024 ± 0.001 (1.091)	1.992 ± 0.063 (1.104)[f]	6.440 ± 0.376 (0.979)[f]	19.270 ± 0.335 (1.179)[d]

Table cont...

Cont.…

Groups	LP σ	LDH φ	NO ψ	ROS Ω
Control	0.853 ± 0.074 (1.000)	0.620 ± 0.072 (1.000)	0.873 ± 0.055 (1.000)	1.000 ± 0.085 (1.000)
DMBA/Croton Oil	1.167 ± 0.074 (1.368)[c]	0.773 ± 0.068 (1.247)	0.935 ± 0.137 (1.071)	1.152 ± 0.034 (1.152)
AI 50 mg/Kg	0.936 ± 0.039 (1.097)[f]	0.714 ± 0.078 (1.151)	0.840 ± 0.062 (0.962)	1.118 ± 0.043 (1.118)
AI 100 mg/Kg	0.877 ± 0.132 (1.201)	0.679 ± 0.078 (1.095)	0.756 ± 0.021 (0.866)[f]	1.009 ± 0.045 (1.009)[f]
AP 50 mg/Kg	0.820 ± 0.206 (0.961)	0.714 ± 0.056 (1.151)	0.966 ± 0.144 (1.107)	1.012 ± 0.076 (1.012)
AP 100 mg/Kg	0.889 ± 0.046 (1.042)[f]	0.653 ± 0.102 (1.053)	0.893 ± 0.238 (1.022)	0.971 ± 0.036 (0.971)[e]
AI/AP-50 mg/Kg	0.765 ± 0.051 (0.897)[e]	0.679 ± 0.022 (1.094)	0.926 ± 0.190 (1.060)	0.928 ± 0.040 (0.928)[e]
AI/AP-100 mg/Kg	0.811 ± 0.060 (0.950)[e]	0.655 ± 0.090 (1.055)	0.821 ± 0.178 (0.941)	1.027 ± 0.093 (1.027)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, σ nmole malondialdehyde formed/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 17: Modulatory influences of FENA in papilloma bearing forestomach tissue against B(a)P induced forestomach papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ
Control	2.462 ± 0.230 (1.000)	0.034 ± 0.003 (1.000)	1.655 ± 0.075 (1.000)	10.767 ± 1.247 (1.000)	17.093 ± 0.169 (1.000)
Benzo(a)pyrene	2.163 ± 0.147 (0.879)	0.024 ± 0.001 (0.709)[c]	1.427 ± 0.051 (0.863)	9.184 ± 0.627 (0.853)	15.705 ± 1.300 (0.919)
50 mg/kg	3.364 ± 0.317 (1.366)[cf]	0.032 ± 0.002 (0.932)[f]	1.845 ± 0.120 (1.115)[f]	10.161 ± 1.081 (0.944)	18.210 ± 1.275 (1.065)
100 mg/kg	3.669 ± 0.355 (1.490)[cf]	0.044 ± 0.002 (1.300)[ce]	1.941 ± 0.068 (1.173)[ce]	10.557 ± 0.679 (0.980)	20.055 ± 1.046 (1.173)[c]

Groups	LP σ	LDH φ	NO ψ	ROS ω
Control	0.666 ± 0.012 (1.000)	0.784 ± 0.050 (1.000)	1.007 ± 0.088 (1.000)	1.000 ± 0.088 (1.000)
Benzo(a)pyrene	0.802 ± 0.047 (1.205)[c]	1.046 ± 0.077 (1.334)[c]	1.111 ± 0.099 (1.103)	1.323 ± 0.093 (1.323)[c]
50 mg/kg	0.700 ± 0.012 (1.050)	0.963 ± 0.083 (1.229)	1.050 ± 0.088 (1.043)	0.871 ± 0.037 (0.871)[cf]
100 mg/kg	0.645 ± 0.026 (0.968)[f]	0.870 ± 0.069 (1.111)	0.933 ± 0.106 (0.927)	0.871 ± 0.074 (0.871)[f]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, σ nmole malondialdehyde formed/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 18: Modulatory influences of FENA on the activity/level of xenobiotic metabolizing enzymes, antioxidants and toxicity related parameters in lung and kidneys of *Swiss albino* mice

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Lung	Control	3.620 ± 0.090 (1.000)	0.029 ± 0.002 (1.000)	1.671 ± 0.124 (1.000)	11.067 ± 0.823 (1.000)	18.060 ± 2.336 (1.000)	0.562 ± 0.046 (1.000)	0.764 ± 0.065 (1.000)
	25 mg/kg	4.211 ± 0.326 (1.163)	0.039 ± 0.001 (1.367)[b]	1.552 ± 0.177 (0.929)	13.252 ± 0.754 (1.197)	18.250 ± 3.982 (1.011)	0.536 ± 0.044 (0.953)	0.690 ± 0.043 (0.903)
	50 mg/kg	4.488 ± 0.576 (1.240)	0.034 ± 0.002 (1.167)	1.583 ± 0.304 (0.948)	17.384 ± 1.234 (1.571)[c]	22.644 ± 2.001 (1.254)	0.499 ± 0.055 (0.888)	0.601 ± 0.031 (0.787)[c]
	100 mg/kg	5.101 ± 0.331 (1.409)[c]	0.036 ± 0.003 (1.239)	1.823 ± 0.269 (1.091)	16.139 ± 0.254 (1.458)[c]	31.184 ± 2.692 (1.727)[c]	0.501 ± 0.069 (0.891)	0.662 ± 0.054 (0.867)

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Kidneys	Control	2.153 ± 0.156 (1.000)	0.026 ± 0.002 (1.000)	1.444 ± 0.085 (1.000)	15.169 ± 0.620 (1.000)	19.123 ± 1.792 (1.000)	0.792 ± 0.061 (1.000)	0.789 ± 0.037 (1.000)
	25 mg/kg	2.494 ± 0.237 (1.158)	0.032 ± 0.002 (1.244)[c]	1.548 ± 0.098 (1.072)	13.838 ± 0.534 (0.912)	22.971 ± 2.067 (1.201)	0.816 ± 0.509 (1.030)	0.777 ± 0.045 (0.985)
	50 mg/kg	3.073 ± 0.347 (1.427)[c]	0.034 ± 0.003 (1.299)[c]	1.948 ± 0.159 (1.349)[c]	18.121 ± 1.179 (1.195)	22.082 ± 2.385 (1.155)	0.768 ± 0.062 (0.970)	0.750 ± 0.038 (0.950)
	100 mg/kg	3.440 ± 0.331 (1.597)[c]	0.034 ± 0.003 (1.325)[c]	1.949 ± 0.059 (1.349)[b]	20.684 ± 1.256 (1.364)[c]	19.892 ± 1.666 (1.040)	0.743 ± 0.058 (0.938)	0.767 ± 0.033 (0.973)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 19: Modulatory influences of FENA on the activity/level of xenobiotic metabolizing enzymes, antioxidants and toxicity related parameters in forestomach and spleen of *Swiss albino* mice

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Forestomach	Control	2.275 ± 0.130 (1.000)	0.027 ± 0.002 (1.000)	1.129 ± 0.157 (1.000)	10.229 ± 1.115 (1.000)	15.181 ± 0.987 (1.000)	0.750 ± 0.067 (1.000)	0.759 ± 0.029 (1.000)
	25 mg/kg	2.750 ± 0.128 (1.209)[c]	0.027 ± 0.001 (1.017)	1.712 ± 0.179 (1.516)[c]	12.609 ± 0.393 (1.233)	14.919 ± 1.356 (0.983)	0.654 ± 0.069 (0.872)	0.739 ± 0.054 (0.974)
	50 mg/kg	3.160 ± 0.201 (1.389)[b]	0.029 ± 0.001 (1.071)	1.682 ± 0.157 (1.489)[c]	17.893 ± 1.805 (1.749)[c]	20.389 ± 1.952 (1.343)[c]	0.640 ± 0.043 (0.853)	0.738 ± 0.019 (0.972)
	100 mg/kg	3.810 ± 0.142 (1.675)[a]	0.027 ± 0.002 (0.992)	1.910 ± 0.075 (1.692)[b]	15.768 ± 1.179 (1.541)[c]	18.376 ± 1.930 (1.210)	0.664 ± 0.062 (0.886)	0.744 ± 0.028 (0.980)

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Spleen	Control	1.389 ± 0.075 (1.000)	0.030 ± 0.002 (1.000)	0.535 ± 0.062 (1.000)	8.089 ± 0.676 (1.000)	12.212 ± 0.610 (1.000)	0.747 ± 0.060 (1.000)	0.789 ± 0.085 (1.000)
	25 mg/kg	1.481 ± 0.072 (1.066)	0.027 ± 0.001 (0.897)	0.617 ± 0.096 (1.154)	8.114 ± 0.473 (1.003)	13.444 ± 2.004 (1.101)	0.774 ± 0.095 (1.036)	0.795 ± 0.083 (1.008)
	50 mg/kg	2.001 ± 0.072 (1.440)[a]	0.032 ± 0.006 (1.080)	0.659 ± 0.023 (1.232)	11.015 ± 0.713 (1.362)[c]	15.777 ± 0.374 (1.263)[b]	0.744 ± 0.042 (0.997)	0.777 ± 0.057 (0.985)
	100 mg/kg	2.005 ± 0.031 (1.443)[a]	0.028 ± 0.002 (0.931)	0.678 ± 0.153 (1.269)	9.624 ± 0.659 (1.190)	13.932 ± 0.699 (1.141)	0.676 ± 0.040 (0.905)	0.787 ± 0.090 (0.998)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 20: Modulatory influences of FENA in lung tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	2.394 ± 0.149 (1.000)	0.033 ± 0.004 (1.000)	1.404 ± 0.009 (1.000)	12.500 ± 1.077 (1.000)	17.918 ± 1.428 (1.000)	0.775 ± 0.065 (1.000)	0.950 ± 0.042 (1.000)
DMBA/Croton Oil	1.909 ± 0.291 (0.797)	0.032 ± 0.001 (0.969)	1.368 ± 0.027 (0.975)	9.677 ± 0.949 (0.774)[c]	15.904 ± 0.816 (0.888)	0.943 ± 0.019 (1.219)	1.080 ± 0.053 (1.137)
AI 50 mg/Kg	2.277 ± 0.237 (0.951)	0.034 ± 0.003 (1.044)	1.644 ± 0.146 (1.171)	10.661 ± 0.393 (0.853)	17.038 ± 1.549 (0.951)	0.922 ± 0.098 (1.190)	0.941 ± 0.047 (0.990)
AI 100 mg/Kg	2.014 ± 0.232 (0.841)	0.036 ± 0.004 (1.115)	1.381 ± 0.064 (0.984)	10.330 ± 0.266 (0.826)	17.631 ± 1.739 (0.984)	0.843 ± 0.072 (1.088)	0.892 ± 0.039 (0.939)[f]
AP 50 mg/Kg	1.890 ± 0.180 (0.789)	0.035 ± 0.003 (1.061)	1.561 ± 0.035 (1.112)	12.717 ± 0.920 (1.017)	16.665 ± 1.264 (0.930)	0.928 ± 0.100 (1.197)	0.996 ± 0.084 (1.048)
AP 100 mg/Kg	2.142 ± 0.211 (0.895)	0.040 ± 0.003 (1.240)[f]	1.635 ± 0.019 (1.165)[ad]	14.079 ± 1.969 (1.126)	17.785 ± 1.275 (0.993)	0.851 ± 0.038 (1.098)	0.975 ± 0.041 (1.026)
AI/AP-50 mg/Kg	2.102 ± 0.034 (0.878)	0.038 ± 0.001 (1.170)[e]	1.835 ± 0.040 (1.307)[bd]	16.490 ± 1.005 (1.319)[ce]	17.205 ± 0.619 (0.960)	0.800 ± 0.037 (1.032)[f]	1.027 ± 0.022 (1.081)
AI/AP-100 mg/Kg	2.114 ± 0.288 (0.883)	0.037 ± 0.002 (1.133)[f]	1.757 ± 0.046 (1.252)[bd]	15.054 ± 0.806 (1.204)[e]	17.795 ± 0.226 (0.993)	0.756 ± 0.036 (0.976)[e]	0.980 ± 0.028 (1.032)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 21: Modulatory influences of FENA in kidneys tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	2.420 ± 0.204 (1.000)	0.026 ± 0.003 (1.000)	1.933 ± 0.053 (1.000)	14.035 ± 1.434 (1.000)	21.939 ± 1.994 (1.000)	1.034 ± 0.012 (1.000)	1.285 ± 0.008 (1.000)
DMBA/Croton Oil	2.121 ± 0.178 (0.876)	0.025 ± 0.002 (0.930)	1.919 ± 0.030 (0.993)	12.389 ± 0.601 (0.883)	17.462 ± 1.207 (0.796)[c]	1.269 ± 0.049 (1.227)[b]	1.442 ± 0.090 (1.122)
AI 50 mg/Kg	2.161 ± 0.081 (0.893)	0.029 ± 0.002 (1.091)	1.936 ± 0.042 (1.002)	12.995 ± 1.563 (0.926)	18.244 ± 0.764 (0.832)	1.112 ± 0.071 (1.076)	1.232 ± 0.009 (0.959)[b]
AI 100 mg/Kg	2.375 ± 0.133 (0.982)	0.032 ± 0.003 (1.214)[f]	2.146 ± 0.079 (1.110)[f]	12.999 ± 0.964 (0.926)	19.052 ± 1.743 (0.868)	1.061 ± 0.117 (1.026)	1.260 ± 0.097 (0.980)
AP 50 mg/Kg	2.270 ± 0.120 (0.938)	0.033 ± 0.004 (1.262)	2.163 ± 0.041 (1.119)[cd]	13.260 ± 1.162 (0.945)	20.891 ± 1.599 (0.952)	1.078 ± 0.065 (1.042)[f]	1.208 ± 0.089 (0.940)
AP 100 mg/Kg	2.322 ± 0.087 (0.960)	0.038 ± 0.004 (1.419)[f]	2.290 ± 0.083 (1.185)[ce]	13.352 ± 1.086 (0.951)	22.383 ± 1.689 (1.020)	0.979 ± 0.076 (0.947)[f]	1.282 ± 0.045 (0.998)
AI/AP-50 mg/Kg	2.543 ± 0.003 (1.051)	0.043 ± 0.001 (1.615)[cd]	2.246 ± 0.035 (1.162)[cd]	13.522 ± 0.430 (0.963)	22.187 ± 0.554 (1.011)[e]	1.071 ± 0.103 (1.036)	1.192 ± 0.034 (0.928)[f]
AI/AP-100 mg/Kg	2.731 ± 0.021 (1.129)[f]	0.043 ± 0.003 (1.609)[ce]	2.456 ± 0.133 (1.271)[be]	15.392 ± 1.170 (1.097)	22.378 ± 1.543 (1.020)[f]	1.021 ± 0.052 (0.988)[f]	1.250 ± 0.026 (0.973)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 22: Modulatory influences of FENA in forestomach tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	2.863 ± 0.107 (1.000)	0.023 ± 0.001 (1.000)	1.759 ± 0.059 (1.000)	10.198 ± 1.401 (1.000)	19.251 ± 2.327 (1.000)	0.761 ± 0.022 (1.000)	1.088 ± 0.082 (1.000)
DMBA/Croton Oil	2.564 ± 0.134 (0.896)	0.021 ± 0.001 (0.916)	1.273 ± 0.048 (0.723)[b]	9.503 ± 0.397 (0.932)	16.135 ± 0.506 (0.838)	0.897 ± 0.082 (1.178)	1.144 ± 0.077 (1.052)
AI 50 mg/Kg	2.731 ± 0.185 (0.954)	0.022 ± 0.002 (0.933)	1.386 ± 0.088 (0.788)[c]	9.780 ± 0.355 (0.959)	19.075 ± 1.755 (0.991)	0.741 ± 0.056 (0.973)	0.999 ± 0.035 (0.919)
AI 100 mg/Kg	2.635 ± 0.247 (0.920)	0.024 ± 0.001 (1.017)	1.369 ± 0.032 (0.778)[b]	10.026 ± 0.837 (0.983)	18.972 ± 1.756 (0.985)	0.631 ± 0.029 (0.829)[c]	1.020 ± 0.074 (0.938)
AP 50 mg/Kg	2.660 ± 0.371 (0.929)	0.021 ± 0.001 (0.923)	1.483 ± 0.063 (0.843)[cf]	9.600 ± 0.828 (0.941)	21.068 ± 2.586 (1.094)[f]	0.661 ± 0.054 (0.868)	0.997 ± 0.085 (0.917)
AP 100 mg/Kg	2.728 ± 0.104 (0.953)	0.022 ± 0.001 (0.965)	1.458 ± 0.046 (0.829)[cf]	10.446 ± 0.712 (1.024)	20.803 ± 1.516 (1.081)[f]	0.645 ± 0.079 (0.848)	1.052 ± 0.132 (0.968)
AI/AP-50 mg/Kg	2.670 ± 0.275 (0.932)	0.022 ± 0.002 (0.948)	1.496 ± 0.087 (0.850)	9.589 ± 0.307 (0.940)	21.338 ± 2.641 (1.108)[f]	0.673 ± 0.030 (0.884)	1.006 ± 0.059 (0.925)
AI/AP-100 mg/Kg	2.935 ± 0.190 (1.025)	0.022 ± 0.002 (0.945)	1.609 ± 0.061 (0.915)[e]	10.340 ± 0.394 (1.014)	19.501 ± 1.400 (1.013)	0.615 ± 0.063 (0.808)[f]	0.934 ± 0.083 (0.859)[f]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue

Table 23: Modulatory influences of FENA in lung tissue against B(a)P induced forestomach papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD Η	CAT κ	LDH φ	NO ψ
Control	2.837 ± 0.271 (1.000)	0.043 ± 0.003 (1.000)	1.961 ± 0.137 (1.000)	13.689 ± 0.992 (1.000)	18.550 ± 1.530 (1.000)	0.879 ± 0.047 (1.000)	1.016 ± 0.033 (1.000)
Benzo(a)pyrene	2.306 ± 0.178 (0.813)	0.033 ± 0.001 (0.768)[c]	1.798 ± 0.149 (0.917)	11.487 ± 0.407 (0.839)	14.853 ± 0.869 (0.801)[c]	1.024 ± 0.074 (1.165)	1.212 ± 0.065 (1.194)
50 mg/kg	2.429 ± 0.214 (0.856)	0.037 ± 0.003 (0.851)	2.275 ± 0.215 (1.160)	14.936 ± 0.311 (1.091)[e]	18.619 ± 1.647 (1.004)	0.971 ± 0.026 (1.105)	0.914 ± 0.083 (0.900)[f]
100 mg/kg	3.307 ± 0.323 (1.166)	0.051 ± 0.004 (1.187)[e]	2.612 ± 0.051 (1.332)[ce]	16.791 ± 0.701 (1.227)[e]	23.196 ± 2.321 (1.250)[f]	0.985 ± 0.093 (1.121)	0.862 ± 0.034 (0.849)[ce]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 24: Modulatory influences of FENA in kidney and spleen tissue against B(a)P induced forestomach papillomagenesis

Tissue	Groups	GST γ	DTD δ	GSH ε	SOD H	CAT K	LDH φ	NO ψ
Kidneys	Control	2.596 ± 0.132 (1.000)	0.036 ± 0.002 (1.000)	1.843 ± 0.052 (1.000)	13.081 ± 0.881 (1.000)	20.527 ± 1.321 (1.000)	1.128 ± 0.093 (1.000)	0.933 ± 0.003 (1.000)
	Benzo(a)pyrene	2.384 ± 0.138 (0.918)	0.027 ± 0.001 (0.736)^{c}	1.772 ± 0.155 (0.961)	11.458 ± 0.633 (0.876)	16.505 ± 1.710 (0.804)^{c}	1.312 ± 0.093 (1.164)	0.999 ± 0.073 (1.071)
	50 mg/kg	2.955 ± 0.126 (1.138)^{f}	0.037 ± 0.003 (1.029)^{f}	2.212 ± 0.178 (1.201)^{f}	14.374 ± 1.179 (1.099)	18.999 ± 0.647 (0.926)	1.102 ± 0.080 (0.977)	0.868 ± 0.023 (0.930)
	100 mg/kg	3.235 ± 0.020 (1.246)^{be}	0.048 ± 0.002 (1.326)^{cd}	2.324 ± 0.107 (1.261)^{cf}	15.347 ± 0.657 (1.173)^{f}	22.846 ± 0.314 (1.113)^{f}	1.075 ± 0.086 (0.953)	0.785 ± 0.034 (0.842)^{cf}

Tissue	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT K	LDH φ	NO ψ
Spleen	Control	1.793 ± 0.111 (1.000)	0.028 ± 0.003 (1.000)	0.909 ± 0.047 (1.000)	8.254 ± 0.433 (1.000)	13.480 ± 0.605 (1.000)	1.031 ± 0.017 (1.000)	1.021 ± 0.033 (1.000)
	Benzo(a)pyrene	1.387 ± 0.091 (0.941)	0.024 ± 0.001 (0.857)	0.836 ± 0.045 (0.920)	7.554 ± 0.271 (0.915)	12.205 ± 0.469 (0.905)	1.317 ± 0.103 (1.277)^{c}	1.287 ± 0.005 (1.260)^{b}
	50 mg/kg	1.794 ± 0.036 (1.000)	0.027 ± 0.002 (0.972)	0.901 ± 0.052 (0.990)	7.792 ± 0.400 (0.944)	14.526 ± 0.316 (1.078)^{f}	1.308 ± 0.034 (1.268)^{b}	1.167 ± 0.048 (1.143)
	100 mg/kg	1.908 ± 0.076 (1.064)	0.033 ± 0.001 (1.169)^{e}	1.065 ± 0.031 (1.172)^{cf}	8.007 ± 0.493 (0.970)	13.791 ± 0.836 (1.023)	1.302 ± 0.069 (1.263)^{c}	1.051 ± 0.056 (1.029)^{f}

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . $^{a}p \le 0.001$, $^{b}p \le 0.01$ and $^{c}p \le 0.05$ represent significant changes against control group. $^{d}p \le 0.001$, $^{e}p \le 0.01$ and $^{f}p \le 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 25: ADME/Tox profile of phytochemicals present in FENA

	Absorption				Distribution		Metabolism		Excretion		Toxicity					
No.	WS	CP	IA	SP	BBBP	CNSP	CYPS	CYPI	TC	ROCTS	Gentox.	MTD	ORAT	ORCT	HepTox	SS
1	-0.326	1.159	95.077	-3.590	-0.226	-2.663	No	No	0.942	No	No	0.905	2.164	2.297	No	No
2	-1.281	1.439	100.000	-2.707	-0.113	-2.784	No	No	0.951	No	No	0.733	2.346	1.853	No	Yes
3	-0.563	1.389	93.243	-2.081	0.818	-1.234	No	1A2	1.678	No	No	1.639	1.819	1.236	No	No
4	-2.859	1.413	92.020	-2.653	-0.150	-2.910	No	No	1.45	No	No	1.166	1.873	12.578	No	Yes
5	-6.364	1.517	94.503	-2.552	0.891	-1.322	No	1A2, C19	1.74	No	No	1.502	1.885	2.998	No	Yes
6	-5.702	1.476	91.551	-2.593	0.028	-1.307	No	1A2	1.565	No	No	1.247	1.916	3.123	No	Yes
7	-6.938	1.423	92.255	-2.385	0.842	-0.995	No	1A2, C19	1.500	No	No	1.527	1.789	1.080	No	Yes
8	-6.729	1.540	94.667	-2.605	0.911	-1.066	No	1A2, C19	1.914	No	No	1.488	1.788	3.003	No	Yes
9	-6.163	1.492	91.219	-2.641	0.024	-1.105	No	1A2	1.692	No	No	1.224	1.849	3.200	No	Yes

Abbreviations: WS: Water solubility (log mol/L), CP: Caco-2 Permeability (log Papp in 10^{-6} cm/s), IA: Intestinal absorption (human) (% Absorbed), SP: Skin Permeability (log Kp), BBBP : Blood-permeability brain barrier P(log BB), CNSP: CNS permeability (log PS), CYPS: CYP450 substrate, CYPI: CYP450 inhibitor, TC: Total Clearance (log ml/min/kg), ROCTS: Renal OCT2 substrate, Gentox: Genotoxicity (AMES), ORCT: Oral Rat Chronic Toxicity (LOAEL) (log mg/kg body weight/day)MTD: Maximum tolerated dose (human) (log mg/kg/day), ORAT: Oral Rat Acute Toxicity (LD50) (mol/kg), HepTox: Hepatotoxicity, SS: Skin Sensitization.

Compounds: 1. 9-Oxa-bicyclo[3.3.1]nonane-1,4-diol, 2. 2,5-Methano-2H-furo[3,2-b]pyran, hexahydro, 3. e-10-Pentadecenol, 4. Dodecanoic acid, 3-hydroxy-, 5. Hexadecanoic acid, ethyl ester, 6. n-Heaxdecanoic acid, 7. Phytol, 8. 9,12-Octadecadienoic acid, ethyl ester and 9. Oleic acid.

6.3.4 Modulatory influence and chemopreventive potential of LEPT

6.3.3.1 General observation and weight gain profile

The body weight of all the experimental animals, at the beginning and end of experiment and relative weight of various organs have been summarized in Table 26-28. The treatment with 50 mg/kg LEPT slightly increased the rate of general body weight gain as compared to negative control group without affecting the relative weight gain profile of various organs [Table 26]. The rate of body weight gain was observed to be significantly decrease in DMBA/croton oil treated group [Table 27]. The treatments with LEPT in pre-initiation and post-initiation phase (100 mg/kg) significantly restored the rate of body weight gain profile [Table 27]. The relative weight gain profile of kidneys was decreased

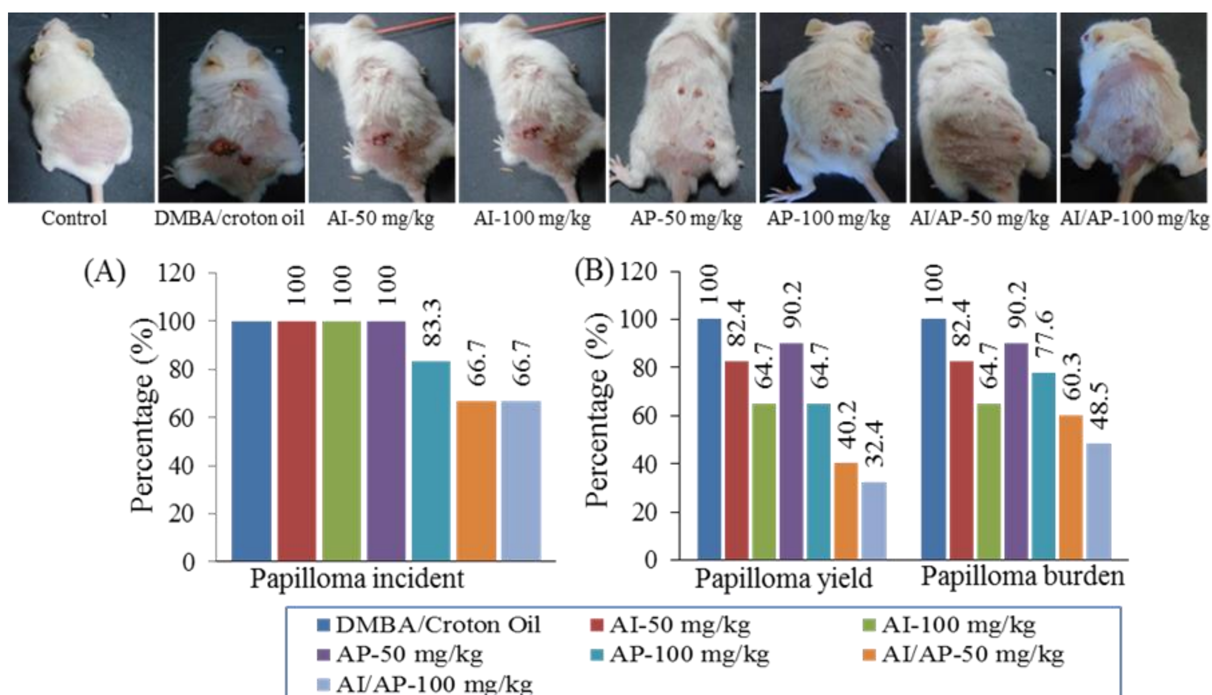


Figure 28: LEPT inhibit the (A) papilloma incidence and (B) papilloma yield and burden against DMBA induced and croton oil promoted skin papillomagenesis: Skin papilloma were induced by DMBA and promoted by croton oil in *Swiss albino* mice. Mice were also fed with LEPT in pre-initiation, post initiation and peri-initiation phase as described in 'Material and Method'. At the end of the experiment photographs of each mouse were taken and papilloma were counted. The upper panel represent the representative photographs of mice from each group**.**

in DMBA/croton oil treated group and treatment with LEPT (50 mg/kg) in pre-initiation phase significantly restored the decreased weight of kidneys [Table 27]. There was also evidence of significant restoration of body weight by LEPT treatment as compared to only B(a)P treated group [Table 28]. The relative weight gain profile of liver, lung and forestomach was found to be significantly increased in B(a)P treated group; however significant restoration in the relative weight gain was observed in lung and forestomach in LEPT treated groups [Table 28].

6.3.3.2 Tumor quantification

The results of chemopreventive potentials of LEPT against DMBA induced and croton oil promoted skin papillomagenesis have been shown in Figure 28. In the present study, treatment with LEPT (50 and 100 mg/kg) in peri-initiation phase decreases the papilloma incidence upto 66.7% (33.3% inhibition) [Figure 28(A)]. Moreover, dose dependent inhibition in terms of papilloma yield and burden were also observed in LEPT treated group across the phases and maximum inhibition (32.4% and 48.5% respectively) was observed in 100 mg/kg LEPT treated in peri-initiation group [Figure 28(B)]. The study

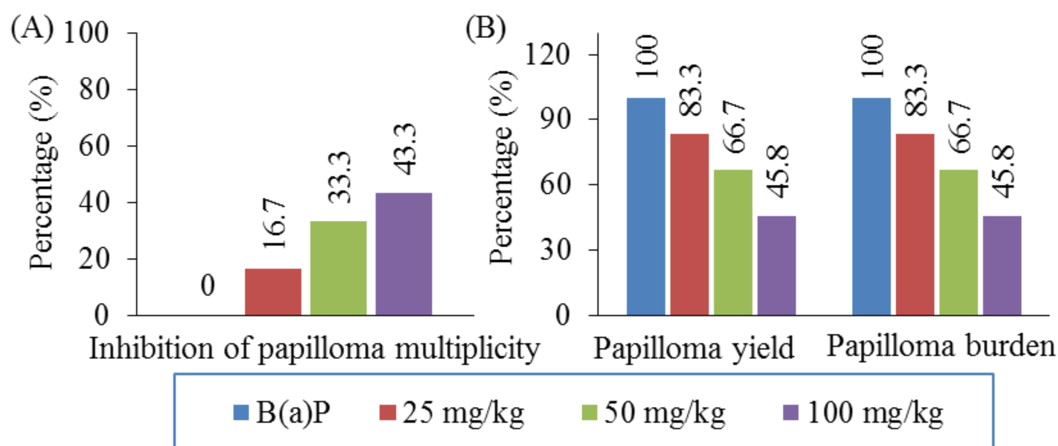


Figure 29: LEPT inhibit the (A) papilloma multiplicity, (B) papilloma yield and burden against B(a)P induced forestomach papillomagenesis: Forestomach papillomas were induced by Benzo(a)pyrene in *Swiss albino* mice and mice were fed with LEPT as described in 'Material and Method'. At the end of the experiment, mice were sacrificed, forestomach were collected and papillomas were counted under magnifying glass.

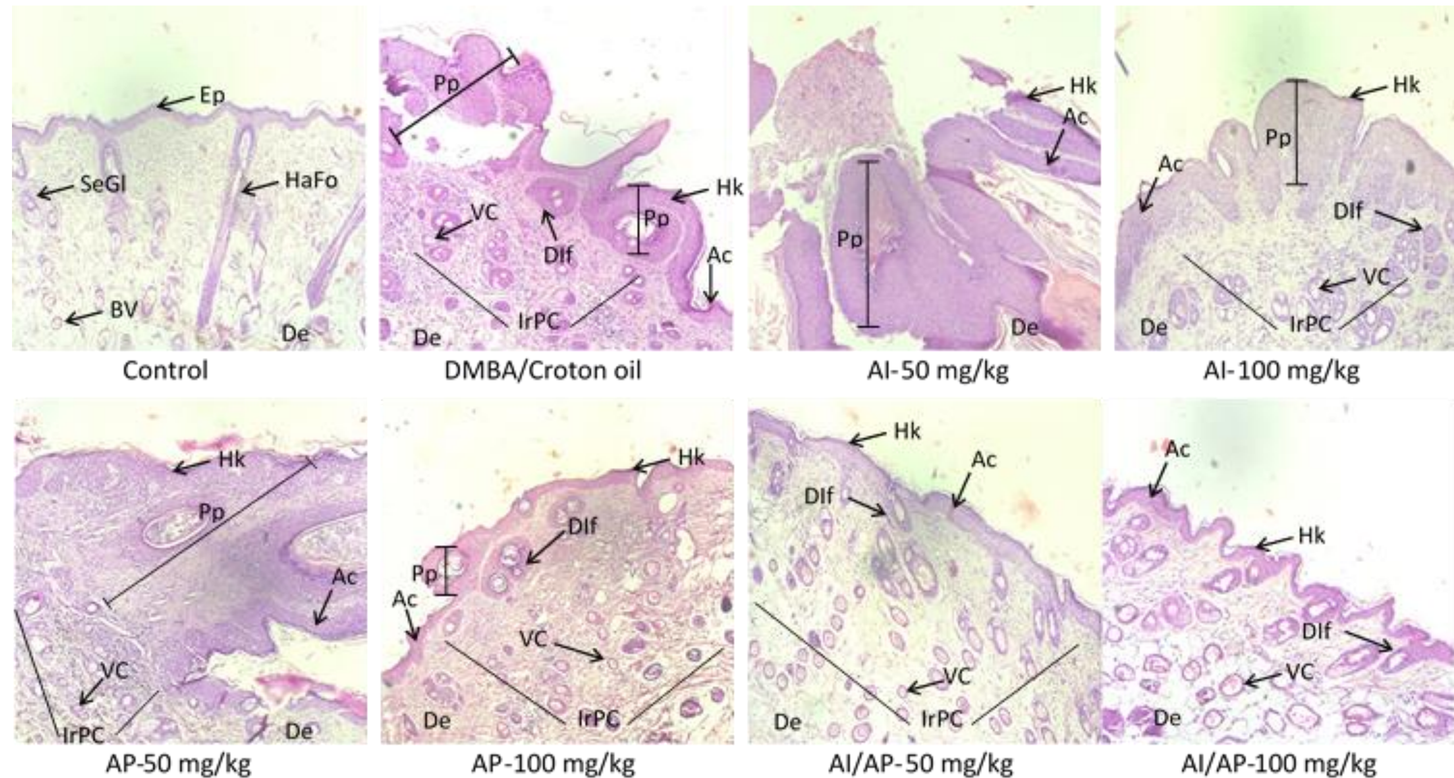


Figure 30: Hematoxylin and eosin stained sections of skin tissues of normal and papilloma bearing mice: Papillomas were chemically induced as described in 'Material and Method'. At the end of the experiment, mice were sacrificed, tissues harvested and processed for microscopy observation as described in 'Material and Method'. The photographs shown (Magnification: 100×) are the representative from each group**.** Abbreviations: Ep: epidermis; HaFo: Hair follicle; SeGl: Sebacious glang; De: Dermis; Ac: Acanthosis; Hk: Hyperkeratosis; Pp: Papillomatosis; Dif: Dermal infiltrations; IrPC: Irregular proliferation of cells and VC: Vascular congestion.

also indicates that oral treatment of mice with LEPT inhibited papilloma multiplicity, yield and burden in dose dependent manner as compared to only B(a)P treated group. The highest dose *i.e.* 100mg/kg inhibited the papilloma multiplicity, papilloma yield and burden upto 43.3%, 45.8% and 45.85 respectively [figure 29].

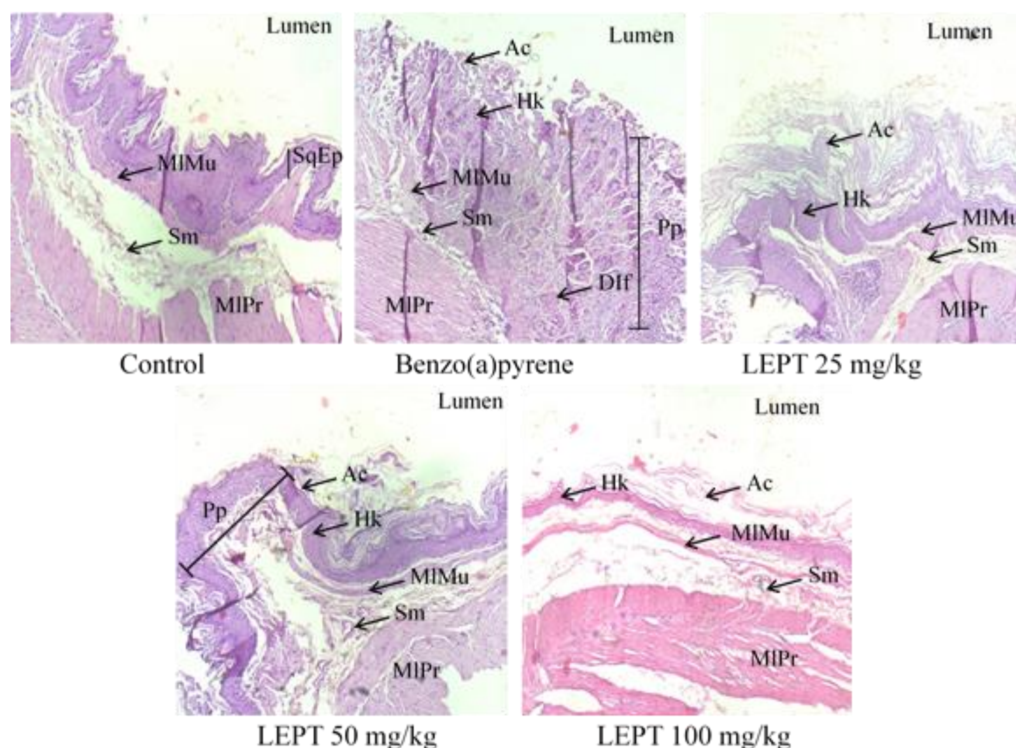


Figure 31: Hematoxylin and eosin stained sections of forestomach tissues of normal and papilloma bearing mice: Papilloma were chemically induced as described in 'Material and Method'. At the end of the experiment, mice were sacrificed, tissues harvested and processed for microscopy observation as described in 'Material and Method'. The photographs shown (Magnification: 100×) are the representative from each group**.** Abbreviations: Ac: Acanthosis; Hk: Hyperkeratosis; Pp: Papillomatosis; Dif: Dermal infiltrations; VC: Vascular congestion; SqEp: Stratified squamous epithelium; MlMu: Mascular mucosae; Sm: Submucosa; MlPr: Mascular propria.

6.3.3.3 Histopathological examination of tumor

The changes in the cellular architecture of normal and papilloma bearing tissues are shown in Figure 30. The histopathology of skin of control group appeared with distinct epidermal and dermal layers. In DMBA/croton oil treated group, skin was showed papilloma related characteristics like papillomatosis, acanthosis, hyperkeratosis, dermal infiltration etc. Treatment of papilloma induced mice with LEPT at different phases of papillomagenesis progressively altered the papilloma related characters. The histology of forestomach of untreated, B(a)P and B(a)P plus LEPT treated mice were shown in Figure 31. The treated dose of LEPT progressively improved the papilloma related distortion of forestomach histological texture as compared to only B(a)P treated group in which severe undifferentiation and distortion exist.

6.3.3.4 Biochemical estimation in liver

The status of the enzymes involved in xenobiotic metabolism, antioxidant and parameters related to toxicity and reactive species were examined in hepatic, extrahepatic as well as papilloma bearing tissues. It is evident from the study that, the treatment of mice with LEPT significantly decreased the activity of Cyt P450R as compared to both the negative control and positive control [Table 29-31]. However, Cytb5R was observed to be decreased significantly only as compared to B(a)P treated group [Table 31]. In contrast to the phase I enzymes, the activity of phase II xenobiotic metabolizing enzymes *i.e.* GST and DTD were significantly induced in comparison to both the carcinogen treated groups [Table 30,31]. Oral treatment of LEPT significantly induced the activities of CAT and GPx as compared to control group [Table 29]. The activities of cellular antioxidants *i.e.* SOD, CAT, GPx and GR were observed to be significantly increased in LEPT treated groups as compared to only carcinogen treated groups [Table 30,31]. Lipid peroxidation in the membranous fraction of liver was found to be significantly increased in both the carcinogen treated groups and treatment with LEPT significantly augmented the level of MDA [Table 30,31]. The specific activity of LDH, the marker of cellular damages were found to be significantly augmented in the LEPT treated groups [Table 29]. The level of reactive species *i.e.* NO and ROS significantly decreased in the LEPT treated groups as compared to carcinogen treated groups [Table 30,31].

6.3.3.5 Biochemical estimation in papilloma bearing tissues

The activities of GST, DTD, SOD, CAT and level of GSH were significantly augmented following LEPT treatment in the papilloma bearing skin samples and forestomach as compared to the carcinogen treated group [Table 32,33]. There was evidence of significant decline in the activity of LDH and levels of LP, NO and ROS following LEPT treatment as compared to only DMBA/croton oil treated group [Table 32,33]. Significant decrease in the activity of LDH and level of LP and ROS were also observed in the forestomach of LEPT treated group as compared to only B(a)P treated group [Table 33].

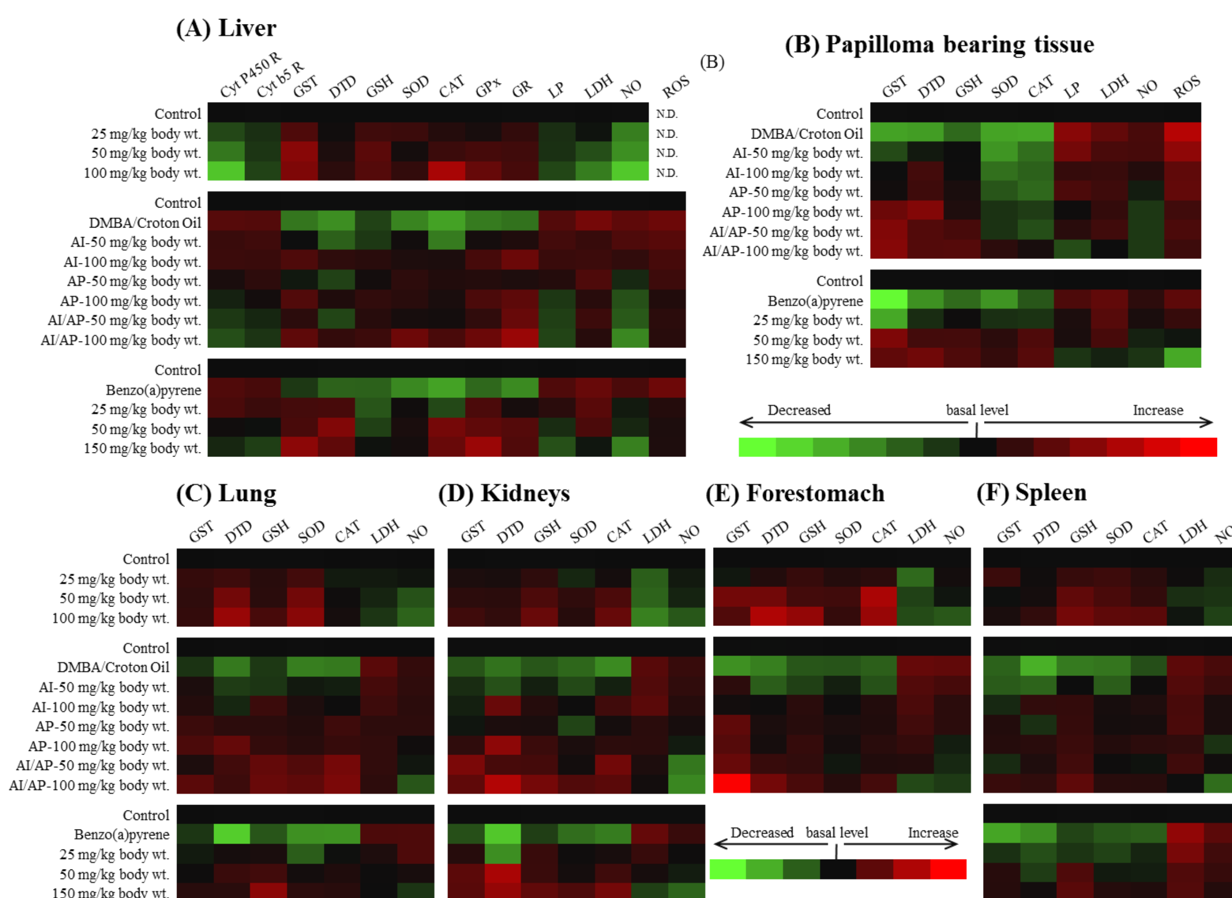


Figure 32: Heat map showing the modulatory influence of LEPT : The activities/levels of enzymes involved in xenobiotic metabolism, antioxidants, toxicity related parameters and reactive species were measured in the hepatic, extra-hepatic as well as papilloma bearing tissues of Swiss albino mice treated with LEPT or carcinogen or LEPT + carcinogen. Values are expressed as mean (N = 4 to 6) fold change in the activity/level in comparison to control.

6.3.3.6 Biochemical estimation in extra-hepatic tissues

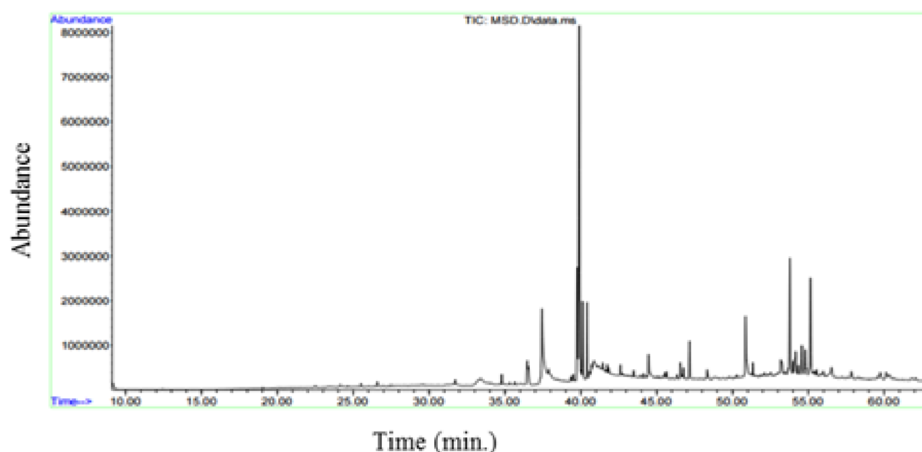
The oral treatment with LEPT was found to be effective in elevating activities/level of DTD and SOD in lung; GSH and CAT in kidneys; GST, DTD, GSH and CAT in forestomach; and GSH, SOD and CAT in spleen [34,35]. There was evidence of significant increase following LEPT treatment in the activities/levels of GST, DTD, GSH, SOD and CAT in lung, kidneys, forestomach and spleen as compared to only DMBA/croton oil treated group [Table 36-39]. LEPT was also found to be effective in significant restoration of activity of LDH and level of NO in lung, kidneys, forestomach and spleen compared to only DMBA/croton oil treated group [Table 36-39]. The treatment with LEPT significantly increases the activities/level of DTD, GSH, SOD and CAT in lung; GST, DTD and GSH in kidneys and spleen as compared to only B(a)P treated group [Table 40-42]; There was also evidence of significant restoration in the level of NO in lung and kidneys; and activities of LDH in kidneys and spleen as compared to only B(a)P treated group [Table 40-42].

6.3.5 Phytochemical characterization of LEPT by GC MS

The phytochemical analysis by GC-MS demonstrated the presence of 42 Nos. of phytochemicals in LEPT. The major phytoconstituents present in LEPT are 9-Octadecenoic acid (Z)-, methyl ester (27.03%), n-Hexadecanoic acid (11.27%), Farnesyl bromide (8.18%), Stigmastan-3,5-diene (7.45%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (6.53%), 13-Docosenamide, (Z)- (5.83), Phytol (3.70%) and Octadecanoic acid, methyl ester(3.66%) [Figure 33]. [Annexure VI]

6.3.6 Pharmacokinetic profile of phytochemicals present in LEPT

The findings of *in silico* ADME/Tox analysis revealed that none of the phytochemicals present in LEPT are genotoxic and hepatotoxic. Most of the phytochemicals resent in LEPT have high intestinal absorption capability (92-95%) and permeability to cells. The phytochemical 9-Octadecenoic acid (Z)-methyl ester, Farnesyl bromide and Stigmastan-3,5-diene can also cross blood brain barrier. The maximum tolerant dose of human, oral chronic (LD50) and acute rat toxicity were found to be considerable moderate range 1.79-2.35 mg/kg/day and 1.08-12.578 mol/kg [Table 43].



Sl N o	RT	Name of compound	Molecular formulae	MW	Peak area (%)
1	36.47	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	1.73
2	37.44	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	11.27
3	39.77	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	6.53
4	39.90	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.48	27.03
5	40.11	Phytol	$C_{20}H_{40}O$	296.53	3.7
6	40.42	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	296.48	3.66
7	44.48	9-Octadecenamide, (Z)-	$C_{18}H_{35}$ NO	281.48	2.13
8	47.18	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390.56	1.95
9	50.87	13-Docosenamide, (Z)-	$C_{22}H_{43}N_{O}$	337.58	5.83
10	53.19	β-Sitosterol	$C_{29}H_{50}O$	414.71	1.19
11	53.79	Farnesyl bromide	$C_{15}H_{25}Br$	285.26	8.18
12	54.01	Germanicol	$C_{30}H_{50}O$	426.70	1.15
13	55.16	Stigmastan-3,5-diene	$C_{29}H_{48}$	396.69	7.45
14	56.55	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	$C_{32}H_{52}O_2$	468.75	1.2
15	59.73	γ-Sitosterol	$C_{29}H_{50}O$	414.71	0.90

Figure 33: Total ion Chromatogram (TIC) of LEPT: GC-MS analysis was performed using DB-5MS (30 x 0.25mm x 0.25 m df, composed of 5% Diphenyl / 95% Dimethyl poly siloxane) column. The major peaks are shown here with the TIC.

Table 26: Effects of LEPT on general body weight gain profile and relative organ weight in untreated *Swiss albino* mice

Groups	Body Weight (gms)		Relative weight of organs				
	Initial	Final	Liver	Lungs	Kidneys	Forestomach	Spleen
Control	22.233 ± 0.858 (1.000)	23.917 ± 0.574 (1.076)	5.485 ± 0.237 (1.000)	0.851 ± 0.078 (1.000)	1.311 ± 0.098 (1.000)	0.352 ± 0.044 (1.000)	0.576 ± 0.049 (1.000)
25 mg/kg body wt./mice/day	21.533 ± 0.634 (1.000)	24.683 ± 0.531 (1.146)[b*]	5.914 ± 0.150 (1.078)	0.755 ± 0.058 (0.888)	1.240 ± 0.051 (0.946)	0.303 ± 0.013 (0.860)	0.701 ± 0.050 (1.217)
50 mg/kg body wt./mice/day	18.550 ± 0.433 (1.000)	22.675 ± 0.729 (1.222)[a*c]	6.281 ± 0.293 (1.145)	0.703 ± 0.045 (0.827)	1.232 ± 0.044 (0.940)	0.291 ± 0.063 (0.827)	0.599 ± 0.061 (1.040)
100 mg/kg body wt./mice/day	20.900 ± 0.980 (1.000)	24.450 ± 1.099 (1.170)[c*]	5.537 ± 0.286 (1.010)	0.739 ± 0.043 (0.868)	1.101 ± 0.058 (0.840)	0.266 ± 0.024 (0.754)	0.500 ± 0.037 (0.867)

Values are expressed as mean ± SEM of 6 animals. Values in parentheses represent relative change in comparison to control mice. [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. '*'mark indicate p value in comparison to initial weight.

Table 27: Effects of LEPT on general body weight gain profile and relative organ weight in DMBA and croton oil treated *Swiss albino* mice

Groups	Body Weight (gms)		Relative weight of organs				
	Initial	Final	Liver	Lungs	Kidneys	Forestomach	Spleen
Control	30.200 ± 1.068 (1.000)	32.690 ± 1.621 (1.082)	6.710 ± 0.926 (1.000)	0.751 ± 0.106 (1.000)	1.383 ± 0.200 (1.000)	0.723 ± 0.076 (1.000)	0.610 ± 0.155 (1.000)
DMBA/Croton Oil	23.283 ± 1.175 (1.000)	20.000 ± 1.633 (0.859)^{c}	7.850 ± 0.641 (1.170)	0.830 ± 0.068 (1.105)	1.130 ± 0.092 (0.817)^{c}	0.690 ± 0.056 (0.955)	0.690 ± 0.056 (1.132)
AI 50 mg/kg body wt./mice/day	25.667 ± 2.178 (1.000)	27.370 ± 2.670 (1.066)	7.799 ± 0.176 (1.162)	0.745 ± 0.012 (0.992)	1.751 ± 0.136 (1.266)^{f}	0.677 ± 0.064 (0.937)	0.727 ± 0.241 (1.193)
AI 100 mg/kg body wt./mice/day	21.229 ± 1.317 (1.000)	28.220 ± 2.304 (1.329)^{c*cf}	7.509 ± 0.613 (1.119)	0.921 ± 0.075 (1.227)	1.375 ± 0.112 (0.994)	0.695 ± 0.057 (0.961)	0.599 ± 0.049 (0.982)
AP 50 mg/kg body wt./mice/day	28.867 ± 1.619 (1.000)	28.970 ± 0.730 (1.004)	7.131 ± 0.726 (1.063)	0.801 ± 0.206 (1.066)	1.598 ± 0.157 (1.155)	0.698 ± 0.076 (0.965)	0.614 ± 0.073 (1.007)
AP 100 mg/kg body wt./mice/day	20.943 ± 0.694 (1.000)	25.383 ± 0.667 (1.212)^{a*e}	8.018 ± 0.955 (1.195)	0.804 ± 0.071 (1.070)	1.471 ± 0.132 (1.063)	0.676 ± 0.055 (0.936)	0.677 ± 0.093 (1.111)
AI/AP 50 mg/kg body wt./mice/day	24.533 ± 3.841 (1.000)	24.030 ± 0.412 (0.979)	7.750 ± 1.037 (1.155)	0.824 ± 0.037 (1.097)	1.479 ± 0.241 (1.069)	0.777 ± 0.158 (1.075)	0.800 ± 0.126 (1.312)
AI/AP 50 mg/kg body wt./mice/day	24.357 ± 2.709 (1.000)	26.453 ± 2.989 (1.086)	7.997 ± 1.143 (1.192)	0.742 ± 0.114 (0.988)	1.575 ± 0.182 (1.138)	0.816 ± 0.213 (1.130)	0.784 ± 0.195 (1.286)

Values are expressed as mean ± SEM of 4-6 animals. Values in parentheses represent relative change in comparison to control mice. $^{a}p\leq0.001$, $^{b}p\leq0.01$ and $^{c}p\leq0.05$ represent significant changes against control group. $^{d}p\leq0.001$, $^{e}p\leq0.01$ and $^{f}p\leq0.05$ represent significant changes against only DMBA/Croton oil treated group. '*'mark indicate p value in comparison to initial weight.

Table 28: Effects of LEPT on general body weight gain profile and relative organ weight in Benzo(a)pyrene treated *Swiss albino* mice

Groups	Body Weight (gms)		Relative weight of organs				
	Initial	Final	Liver	Lungs	Kidneys	Forestomach	Spleen
Control	21.200 ± 1.188 (1.000)	22.335 ± 0.305 (1.054)	6.457 ± 1.106 (1.000)	1.133 ± 0.113 (1.000)	1.503 ± 0.230 (1.000)	0.777 ± 0.093 (1.000)	0.546 ± 0.149 (1.000)
Benzo(a)pyrene	24.580 ± 0.651 (1.000)	19.060 ± 1.556 (0.775)[c*b]	8.200 ± 0.670 (1.270)[c]	1.443 ± 0.118 (1.273)[c]	1.905 ± 0.156 (1.267)	1.018 ± 0.083 (1.309)[c]	0.514 ± 0.042 (0.941)
25 mg/kg body wt./mcie/day	22.350 ± 0.969 (1.000)	25.560 ± 2.087 (1.144)[f]	6.897 ± 0.563 (1.068)	1.189 ± 0.097 (1.050)	1.530 ± 0.125 (1.018)	0.806 ± 0.066 (1.037)	0.630 ± 0.051 (1.152)
50 mg/kg body wt./mcie/day	26.857 ± 2.217 (1.000)	25.040 ± 2.045 (0.932)	7.021 ± 0.573 (1.087)	1.206 ± 0.098 (1.064)	1.506 ± 0.123 (1.002)	0.751 ± 0.061 (0.966)[f]	0.583 ± 0.048 (1.067)
100 mg/kg body wt./mcie/day	27.786 ± 1.456 (1.000)	29.635 ± 1.367 (1.067)[f]	7.273 ± 1.032 (1.126)	1.064 ± 0.090 (0.939)[f]	1.377 ± 0.084 (0.916)[f]	0.724 ± 0.040 (0.931)[f]	0.564 ± 0.083 (1.032)

Values are expressed as mean ± SEM of 4-6 animals. Values in parentheses represent relative change in comparison to control mice. [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only Benzo(a)pyrene treated group. '*'mark indicate p value in comparison to initial weight.

Table 29: Modulatory influences of LEPT on the activity/level of xenobiotic metabolizing enzymes, antioxidants and toxicity related parameters in hepatic tissues of *Swiss albino* mice

Groups	Cyt P_{450} R α	Cyt b_5 R β	GST γ	DTD δ	GSH ε	SOD η
Control	0.349 ± 0.027 (1.000)	0.852 ± 0.045 (1.000)	4.867 ± 0.413 (1.000)	0.035 ± 0.004 (1.000)	4.123 ± 0.420 (1.000)	20.635 ± 1.181 (1.000)
25 mg/kg	0.321 ± 0.027 (0.921)	0.813 ± 0.040 (0.954)	5.775 ± 0.232 (1.187)	0.036 ± 0.002 (1.013)	4.714 ± 0.345 (1.143)	23.345 ± 0.375 (1.131)
50 mg/kg	0.299 ± 0.018 (0.857)	0.807 ± 0.038 (0.947)	6.574 ± 0.409 (1.351)[c]	0.037 ± 0.004 (1.046)	5.031 ± 0.306 (1.220)	21.144 ± 0.627 (1.025)
100 mg/kg	0.261 ± 0.017 (0.750)[c]	0.791 ± 0.033 (0.928)	6.443 ± 0.328 (1.324)[c]	0.039 ± 0.004 (1.087)	4.968 ± 0.496 (1.205)	22.827 ± 1.394 (1.106)

Groups	CAT κ	GPx π	GR π	LP σ	LDH φ	NO ψ
Control	28.433 ± 1.523 (1.000)	26.439 ± 1.117 (1.000)	21.903 ± 2.187 (1.000)	0.819 ± 0.027 (1.000)	1.181 ± 0.098 (1.000)	1.143 ± 0.098 (1.000)
25 mg/kg	30.349 ± 1.688 (1.067)	27.279 ± 1.496 (1.032)	24.261 ± 2.519 (1.108)	0.783 ± 0.030 (0.955)	1.172 ± 0.057 (0.993)	0.967 ± 0.030 (0.846)
50 mg/kg	32.163 ± 3.111 (1.131)	30.667 ± 2.152 (1.160)	25.103 ± 1.985 (1.146)	0.781 ± 0.042 (0.953)	1.077 ± 0.032 (0.912)	0.941 ± 0.069 (0.823)
100 mg/kg	41.061 ± 2.876 (1.444)[b]	33.155 ± 2.731 (1.254)[c]	25.514 ± 1.046 (1.165)	0756 ± 0.044 (0.923)	1.002 ± 0.104 (0.848)	0.853 ± 0.029 (0.746)[c]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . $^a p \leq 0.001$, $^b p \leq 0.01$ and $^c p \leq 0.05$ represent significant changes against control group. α µmole of NADPH oxidized/min/mg protein, β µmole of NADH oxidized/min/mg protein, γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, π nmole of NADPH consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 30: Modulatory influences of LEPT in hepatic tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	Cyt P_{450} R α	Cyt b_5 R β	GST γ	DTD δ	GSH ε	SOD η	CAT κ
Control	0.349 ± 0.016 (1.000)	0.801 ± 0.076 (1.000)	4.236 ± 0.344 (1.000)	0.041 ± 0.003 (1.000)	3.857 ± 0.405 (1.000)	22.322 ± 0.985 (1.000)	27.377 ± 1.987 (1.000)
DMBA/Croton Oil	0.422 ± 0.012 (1.210)^{c}	0.961 ± 0.073 (1.200)	3.630 ± 0.004 (0.857)	0.034 ± 0.002 (0.825)	3.584 ± 0.378 (0.929)	18.702 ± 0.498 (0.838)^{c}	21.843 ± 1.446 (0.798)^{c}
AI 50 mg/Kg	0.393 ± 0.018 (1.126)	0.917 ± 0.015 (1.144)	4.232 ± 0.002 (0.999)^{d}	0.036 ± 0.003 (0.888)	3.633 ± 0.075 (0.942)	22.644 ± 1.891 (1.014)	23.284 ± 1.918 (0.850)
AI 100 mg/Kg	0.395 ± 0.004 (1.134)	0.903 ± 0.077 (1.126)	5.029 ± 0.232 (1.187)^{e}	0.045 ± 0.004 (1.097)^{f}	4.174 ± 0.355 (1.082)	23.413 ± 1.641 (1.049)	28.603 ± 0.964 (1.045)^{f}
AP 50 mg/Kg	0.362 ± 0.016 (1.038)^{f}	0.876 ± 0.059 (1.093)	4.175 ± 0.343 (0.986)	0.038 ± 0.002 (0.929)	3.938 ± 0.166 (1.021)	24.397 ± 0.338 (1.093)^{d}	28.944 ± 1.900 (1.057)^{f}
AP 100 mg/Kg	0.339 ± 0.027 (0.973)^{f}	0.818 ± 0.047 (1.021)	5.067 ± 0.484 (1.196)^{f}	0.043 ± 0.002 (1.053)^{f}	4.258 ± 0.182 (1.104)	24.159 ± 1.390 (1.082)^{e}	28.443 ± 1.920 (1.039)^{f}
AI/AP-50 mg/Kg	0.328 ± 0.029 (0.942)^{f}	0.775 ± 0.026 (0.967)	4.601 ± 0.448 (1.086)	0.038 ± 0.002 (0.924)	4.146 ± 0.385 (1.075)	22.965 ± 1.249 (1.029)^{f}	27.935 ± 1.228 (1.020)^{f}
AI/AP-100 mg/Kg	0.318 ± 0.020 (0.912)^{f}	0.759 ± 0.072 (0.948)	5.219 ± 0.426 (1.232)^{f}	0.046 ± 0.004 (1.108)^{f}	4.383 ± 0.444 (1.136)	28.462 ± 1.514 (1.275)^{ce}	32.136 ± 3.295 (1.174)^{f}

Table cont...

Cont….

Groups	GPx π	GR π	LP Σ	LDH Φ	NO ψ	ROS ω
Control	0.814 ± 0.052 (1.000)	1.293 ± 0.044 (1.000)	1.022 ± 0.049 (1.000)	1.000 ± 0.045 (1.000)	0.814 ± 0.052 (1.000)	1.293 ± 0.044 (1.000)
DMBA/Croton Oil	0.978 ± 0.050 (1.201)^{c}	1.677 ± 0.028 (1.297)^{a}	1.261 ± 0.025 (1.234)^{c}	1.272 ± 0.038 (1.272)^{b}	0.978 ± 0.050 (1.201)^{c}	1.677 ± 0.028 (1.297)^{a}
AI 50 mg/Kg	0.941 ± 0.025 (1.156)	1.450 ± 0.094 (1.121)	1.214 ± 0.024 (1.188)^{c}	1.212 ± 0.081 (1.212)	0.941 ± 0.025 (1.156)	1.450 ± 0.094 (1.121)
AI 100 mg/Kg	0.917 ± 0.039 (1.126)	1.467 ± 0.065 (1.134)^{f}	1.128 ± 0.015 (1.103)^{f}	1.164 ± 0.101 (1.164)	0.917 ± 0.039 (1.126)	1.467 ± 0.065 (1.134)^{f}
AP 50 mg/Kg	0.870 ± 0.014 (1.068)	1.534 ± 0.030 (1.186)^{bf}	0.986 ± 0.025 (0.965)^{e}	1.139 ± 0.060 (1.139)	0.870 ± 0.014 (1.068)	1.534 ± 0.030 (1.186)^{bf}
AP 100 mg/Kg	0.768 ± 0.050 (0.943)^{f}	1.396 ± 0.104 (1.079)^{f}	0.929 ± 0.009 (0.909)^{e}	1.058 ± 0.043 (1.058)^{e}	0.768 ± 0.050 (0.943)^{f}	1.396 ± 0.104 (1.079)^{f}
AI/AP- 50 mg/Kg	0.760 ± 0.054 (0.933)^{f}	1.467 ± 0.035 (1.134)^{ce}	0.916 ± 0.008 (0.897)^{e}	1.087 ± 0.061 (1.087)	0.760 ± 0.054 (0.933)^{f}	1.467 ± 0.035 (1.134)^{ce}
AI/AP- 100 mg/Kg	0.754 ± 0.004 (0.926)^{f}	1.391 ± 0.065 (1.076)^{f}	0.853 ± 0.021 (0.834)^{cd}	1.078 ± 0.024 (1.078)^{f}	0.754 ± 0.004 (0.926)^{f}	1.391 ± 0.065 (1.076)^{f}

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. $^{d}p \leq 0.001$, $^{e}p \leq 0.01$ and $^{f}p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. α µmole of NADPH oxidized/min/mg protein, β µmole of NADH oxidized/min/mg protein, γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, π nmole of NADPH consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 31: Modulatory influences of LEPT in hepatic tissue against B(a)P induced forestomach papillomagenesis

Groups	Cyt P_{450} R α	Cyt b_5 R β	GST γ	DTD δ	GSH Ε	SOD η	CAT κ
Control	0.359 ± 0.023 (1.000)	0.776 ± 0.022 (1.000)	4.551 ± 0.171 (1.000)	0.037 ± 0.005 (1.000)	3.509 ± 0.182 (1.000)	22.299 ± 0.750 (1.000)	28.049 ± 1.100 (1.000)
Benzo(a)pyrene	0.429 ± 0.007 $(1.194)^{c}$	0.905 ± 0.015 $(1.167)^{b}$	4.285 ± 0.556 (0.942)	0.033 ± 0.001 (0.885)	3.112 ± 0.170 (0.887)	18.579 ± 1.021 $(0.833)^{c}$	22.358 ± 0.670 $(0.797)^{b}$
25 mg/kg	0.422 ± 0.019 (1.174)	0.876 ± 0.087 (1.130)	5.268 ± 0.205 (1.157)	0.043 ± 0.000 $(1.166)^{e}$	3.170 ± 0.123 (0.903)	22.603 ± 0.843 $(1.014)^{f}$	25.825 ± 2.307 (0.921)
50 mg/kg	0.364 ± 0.019 $(1.012)^{f}$	0.773 ± 0.077 (0.997)	5.327 ± 0.222 $(1.170)^{cf}$	0.049 ± 0.001 $(1.331)^{ce}$	3.266 ± 0.183 (0.931)	23.177 ± 1.021 $(1.039)^{f}$	36.411 ± 1.902 $(1.298)^{ce}$
100 mg/kg	0.347 ± 0.015 $(0.966)^{e}$	0.725 ± 0.056 $(0.934)^{f}$	6.208 ± 0.601 $(1.364)^{cf}$	0.046 ± 0.004 $(1.235)^{f}$	3.489 ± 0.071 (0.994)	22.838 ± 0.557 $(1.024)^{f}$	35.336 ± 2.611 $(1.260)^{cf}$

Table cont...

Cont.…

Groups	GPx π	GR π	LP Σ	LDH Φ	NO ψ	ROS ω
Control	22.962 ± 1.061 (1.000)	19.106 ± 0.851 (1.000)	0.821 ± 0.025 (1.000)	1.308 ± 0.038 (1.000)	1.184 ± 0.092 (1.000)	1.000 ± 0.122 (1.000)
Benzo(a)pyrene	20.119 ± 0.685 (0.876)	15.852 ± 0.119 (0.830)[c]	0.977 ± 0.048 (1.190)	1.645 ± 0.085 (1.258)[c]	1.393 ± 0.060 (1.176)	1.276 ± 0.078 (1.276)[c]
25 mg/kg	27.043 ± 0.629 (1.178)[ce]	19.703 ± 0.610 (1.031)[e]	0.891 ± 0.048 (1.085)	1.591 ± 0.094 (1.216)	1.164 ± 0.076 (0.983)	1.067 ± 0.009 (1.067)
50 mg/kg	28.391 ± 1.263 (1.236)[ce]	22.990 ± 1.041 (1.203)[ce]	0.787 ± 0.047 (0.959)[f]	1.541 ± 0.052 (1.178)[c]	1.145 ± 0.088 (0.967)	1.047 ± 0.007 (1.047)
100 mg/kg	32.221 ± 1.587 (1.403)[be]	22.792 ± 0.913 (1.193)[ce]	0.746 ± 0.053 (0.909)[f]	1.294 ± 0.096 (0.989)[f]	1.000 ± 0.083 (0.844)[f]	1.050 ± 0.018 (1.050)[f]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. α µmole of NADPH oxidized/min/mg protein, β µmole of NADH oxidized/min/mg protein, γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, π nmole of NADPH consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 32: Modulatory influences of LEPT in papilloma bearing tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT K
Control	0.935 ± 0.016 (1.000)	0.025 ± 0.002 (1.000)	1.100 ± 0.082 (1.000)	8.051 ± 0.607 (1.000)	13.424 ± 1.319 (1.000)
DMBA/Croton Oil	0.746 ± 0.038 (0.798)[c]	0.020 ± 0.000 (0.804)[c]	0.963 ± 0.041 (0.875)	6.408 ± 0.108 (0.796)[c]	10.625 ± 0.437 (0.792)[c]
AI 50 mg/Kg	0.858 ± 0.010 (0.918)[cf]	0.025 ± 0.002 (0.985)	1.103 ± 0.081 (1.002)	6.563 ± 0.646 (0.815)	11.653 ± 0.141 (0.868)
AI 100 mg/Kg	0.934 ± 0.013 (0.999)[e]	0.029 ± 0.003 (1.156)[f]	1.106 ± 0.041 (1.005)	6.972 ± 0.141 (0.866)[f]	11.901 ± 1.223 (0.887)
AP 50 mg/Kg	0.949 ± 0.077 (1.015)	0.029 ± 0.002 (1.142)[f]	1.144 ± 0.139 (1.039)	7.269 ± 0.157 (0.903)[f]	11.764 ± 0.604 (0.876)
AP 100 mg/Kg	1.188 ± 0.108 (1.271)[e]	0.034 ± 0.002 (1.337)[ce]	1.158 ± 0.060 (1.052)[f]	7.649 ± 0.326 (0.950)[f]	12.575 ± 0.849 (0.937)
AI/AP-50 mg/Kg	1.244 ± 0.084 (1.331)[ce]	0.030 ± 0.001 (1.198)[ce]	1.285 ± 0.112 (1.167)	7.649 ± 0.243 (0.950)[e]	12.241 ± 0.827 (0.912)
AI/AP-100 mg/Kg	1.259 ± 0.099 (1.347)[e]	0.030 ± 0.001 (1.201)[ce]	1.329 ± 0.125 (1.208)[f]	8.765 ± 0.185 (1.089)[d]	13.869 ± 0.944 (1.033)[f]

Table cont...

Cont….

Groups	LP σ	LDH Φ	NO ψ	ROS ω
Control	0.728 ± 0.062 (1.000)	0.937 ± 0.052 (1.000)	0.852 ± 0.002 (1.000)	1.000 ± 0.046 (1.000)
DMBA/Croton Oil	0.984 ± 0.042 (1.352)^{c}	1.165 ± 0.039 (1.243)^{c}	0.996 ± 0.011 (1.170)^{b}	1.483 ± 0.078 (1.483)^{b}
AI 50 mg/Kg	0.944 ± 0.055 (1.296)^{c}	1.098 ± 0.072 (1.172)	0.991 ± 0.043 (1.164)	1.371 ± 0.013 (1.371)^{b}
AI 100 mg/Kg	0.813 ± 0.019 (1.117)^{f}	1.054 ± 0.104 (1.125)	0.906 ± 0.065 (1.064)	1.236 ± 0.013 (1.236)^{cf}
AP 50 mg/Kg	0.861 ± 0.055 (1.182)	1.069 ± 0.070 (1.141)	0.835 ± 0.043 (0.980)^{f}	1.243 ± 0.133 (1.243)
AP 100 mg/Kg	0.733 ± 0.032 (1.007)^{e}	1.040 ± 0.048 (1.110)	0.804 ± 0.035 (0.944)^{e}	1.146 ± 0.034 (1.146)^{cf}
AI/AP-50 mg/Kg	0.788 ± 0.071 (1.083)	1.076 ± 0.026 (1.148)	0.803 ± 0.061 (0.943)^{f}	1.178 ± 0.035 (1.178)^{cf}
AI/AP-100 mg/Kg	0.665 ± 0.070 (0.914)^{f}	0.938 ± 0.046 (1.001)^{f}	0.801 ± 0.022 (0.940)^{e}	1.139 ± 0.040 (1.139)^{f}

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. $^{d}p \leq 0.001$, $^{e}p \leq 0.01$ and $^{f}p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, σ nmole malondialdehyde formed/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 33: Modulatory influences of LEPT in papilloma bearing forestomach tissue against B(a)P induced forestomach papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT K
Control	2.172 ± 0.171 (1.000)	0.026 ± 0.002 (1.000)	1.462 ± 0.060 (1.000)	12.571 ± 0.662 (1.000)	18.855 ± 1.516 (1.000)
Benzo(a)pyrene	1.468 ± 0.012 (0.676)^{c}	0.021 ± 0.001 (0.821)	1.277 ± 0.003 (0.873)	10.258 ± 0.424 (0.816)^{c}	16.994 ± 1.151 (0.901)
25 mg/kg	1.715 ± 0.031 (0.790)^{e}	0.025 ± 0.000 (0.959)^{f}	1.471 ± 0.111 (1.006)	11.994 ± 0.845 (0.954)	17.892 ± 1.427 (0.949)
50 mg/kg	2.876 ± 0.173 (1.324)^{ce}	0.030 ± 0.001 (1.155)^{e}	1.693 ± 0.099 (1.158)^{f}	13.650 ± 1.292 (1.086)	22.394 ± 1.131 (1.188)^{f}
100 mg/kg	2.689 ± 0.063 (1.238)^{cd}	0.033 ± 0.004 (1.285)^{f}	1.736 ± 0.077 (1.187)^{ce}	13.984 ± 1.215 (1.112)^{f}	22.799 ± 1.680 (1.209)^{f}

Table cont...

Cont.…

Groups	LP σ	LDH Φ	NO ψ	ROS ω
Control	0.622 ± 0.058 (1.000)	0.877 ± 0.059 (1.000)	1.004 ± 0.047 (1.000)	1.000 ± 0.073 (1.000)
Benzo(a)pyrene	0.735 ± 0.041 (1.183)	1.088 ± 0.022 (1.241)[c]	1.098 ± 0.064 (1.098)	1.228 ± 0.082 (1.228)
25 mg/kg	0.650 ± 0.036 (1.045)	1.060 ± 0.009 (1.209)	1.044 ± 0.049 (1.039)	1.120 ± 0.094 (1.120)
50 mg/kg	0.645 ± 0.021 (1.037)	1.023 ± 0.047 (1.167)	0.977 ± 0.009 (0.973)	0.989 ± 0.006 (0.989)[f]
100 mg/kg	0.589 ± 0.020 (0.947)[f]	0.853 ± 0.016 (0.973)[e]	0.951 ± 0.025 (0.947)	0.788 ± 0.030 (0.788)[cf]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, σ nmole malondialdehyde formed/mg protein, φ µmole of NADH oxidized/min/mg protein, ψ nmole NO/g tissue and ω relative fluorescence unit (RFU).

Table 34: Modulatory influences of LEPT on the activity/level of xenobiotic metabolizing enzymes, antioxidants and toxicity related parameters in lung and kidneys of *Swiss albino* mice

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Lung	Control	2.938 ± 0.135 (1.000)	0.031 ± 0.004 (1.000)	1.957 ± 0.120 (1.000)	14.316 ± 0.451 (1.000)	15.735 ± 0.625 (1.000)	0.933 ± 0.033 (1.000)	0.863 ± 0.017 (1.000)
	25 mg/kg	3.265 ± 0.175 (1.111)	0.036 ± 0.003 (1.138)	2.112 ± 0.085 (1.079)	16.499 ± 0.731 (1.153)[c]	15.475 ± 0.567 (0.983)	0.918 ± 0.055 (0.985)	0.856 ± 0.076 (0.991)
	50 mg/kg	3.229 ± 0.253 (1.099)	0.040 ± 0.002 (1.289)[c]	2.122 ± 0.114 (1.084)	18.406 ± 0.535 (1.286)[a]	15.880 ± 0.571 (1.009)	0.904 ± 0.016 (0.970)	0.783 ± 0.064 (0.907)
	100 mg/kg	3.260 ± 0.185 (1.109)	0.044 ± 0.003 (1.397)[c]	2.283 ± 0.129 (1.166)	19.338 ± 1.166 (1.351)[b]	16.140 ± 0.810 (1.026)	0.883 ± 0.054 (0.947)	0.762 ± 0.057 (0.883)

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Kidneys	Control	2.459 ± 0.114 (1.000)	0.035 ± 0.002 (1.000)	2.095 ± 0.109 (1.000)	16.774 ± 1.141 (1.000)	19.459 ± 1.762 (1.000)	0.947 ± 0.093 (1.000)	0.911 ± 0.014 (1.000)
	25 mg/kg	2.571 ± 0.193 (1.045)	0.036 ± 0.002 (1.035)	2.293 ± 0.209 (1.095)	16.222 ± 0.894 (0.967)	19.908 ± 0.459 (1.023)	0.843 ± 0.058 (0.890)	0.896 ± 0.036 (0.984)
	50 mg/kg	2.677 ± 0.114 (1.088)	0.038 ± 0.001 (1.084)	2.472 ± 0.234 (1.180)	18.432 ± 1.344 (1.099)	22.934 ± 1.851 (1.179)	0.837 ± 0.042 (0.884)	0.884 ± 0.040 (0.970)
	100 mg/kg	2.812 ± 0.222 (1.143)	0.039 ± 0.003 (1.103)	2.642 ± 0.170 (1.261)[c]	17.996 ± 1.402 (1.073)	24.421 ± 1.131 (1.255)[c]	0.799 ± 0.042 (0.844)	0.820 ± 0.038 (0.900)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 35: Modulatory influences of LEPT on the activity/level of xenobiotic metabolizing enzymes, antioxidants and toxicity related parameters in forestomach and spleen of *Swiss albino* mice

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Forestomach	Control	2.152 ± 0.207 (1.000)	0.027 ± 0.003 (1.000)	1.517 ± 0.089 (1.000)	11.514 ± 0.436 (1.000)	17.617 ± 1.423 (1.000)	0.959 ± 0.067 (1.000)	0.887 ± 0.023 (1.000)
	25 mg/kg	2.124 ± 0.214 (0.987)	0.029 ± 0.002 (1.060)	1.695 ± 0.060 (1.117)	12.291 ± 0.416 (1.067)	19.145 ± 1.002 (1.087)	0.839 ± 0.044 (0.875)	0.907 ± 0.070 (1.022)
	50 mg/kg	2.774 ± 0.228 (1.289)[c]	0.035 ± 0.002 (1.287)[c]	1.753 ± 0.068 (1.155)	13.206 ± 0.652 (1.147)	25.539 ± 2.245 (1.450)[c]	0.892 ± 0.034 (0.930)	0.878 ± 0.038 (0.990)
	100 mg/kg	2.569 ± 0.166 (1.194)[c]	0.040 ± 0.002 (1.463)[b]	2.101 ± 0.113 (1.384)[b]	12.823 ± 0.568 (1.114)	24.624 ± 2.013 (1.398)[c]	0.878 ± 0.040 (0.916)	0.797 ± 0.057 (0.899)

	Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Spleen	Control	1.602 ± 0.150 (1.000)	0.030 ± 0.001 (1.000)	0.992 ± 0.026 (1.000)	8.071 ± 0.587 (1.000)	11.049 ± 0.930 (1.000)	1.207 ± 0.023 (1.000)	0.868 ± 0.028 (1.000)
	25 mg/kg	1.811 ± 0.162 (1.130)	0.030 ± 0.002 (1.014)	1.110 ± 0.082 (1.118)	9.193 ± 0.444 (1.139)	11.936 ± 1.063 (1.080)	1.218 ± 0.046 (1.009)	0.832 ± 0.026 (0.959)
	50 mg/kg	1.598 ± 0.086 (0.998)	0.030 ± 0.001 (1.023)	1.229 ± 0.071 (1.238)[c]	9.461 ± 0.535 (1.172)	12.246 ± 1.020 (1.108)	1.153 ± 0.066 (0.955)	0.823 ± 0.024 (0.949)
	100 mg/kg	1.666 ± 0.155 (1.040)	0.033 ± 0.003 (1.105)	1.284 ± 0.115 (1.294)[c]	9.940 ± 0.476 (1.232)[c]	13.501 ± 0.536 (1.222)[c]	1.195 ± 0.056 (0.990)	0.807 ± 0.020 (0.930)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 36: Modulatory influences of LEPT in lung tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH Φ	NO ψ
Control	2.747 ± 0.200 (1.000)	0.032 ± 0.001 (1.000)	2.012 ± 0.135 (1.000)	15.174 ± 0.923 (1.000)	17.079 ± 1.451 (1.000)	0.802 ± 0.080 (1.000)	1.097 ± 0.024 (1.000)
DMBA/Croton Oil	2.606 ± 0.222 (0.949)	0.027 ± 0.001 (0.853)^{c}	1.896 ± 0.149 (0.943)	12.843 ± 1.027 (0.846)	14.564 ± 0.465 (0.853)	0.976 ± 0.029 (1.217)	1.218 ± 0.078 (1.110)
AI 50 mg/Kg	2.871 ± 0.284 (1.045)	0.030 ± 0.001 (0.937)^{f}	1.904 ± 0.085 (0.947)	14.939 ± 1.328 (0.984)	16.703 ± 1.523 (0.978)	0.931 ± 0.025 (1.161)	1.204 ± 0.082 (1.098)
AI 100 mg/Kg	2.924 ± 0.063 (1.064)	0.031 ± 0.001 (0.967)^{f}	2.277 ± 0.081 (1.132)	15.869 ± 1.243 (1.046)	17.159 ± 0.909 (1.005)	0.914 ± 0.018 (1.140)	1.191 ± 0.048 (1.086)
AP 50 mg/Kg	3.109 ± 0.319 (1.132)	0.035 ± 0.001 (1.090)^{e}	2.187 ± 0.205 (1.087)	16.142 ± 0.318 (1.064)f	19.503 ± 1.193 (1.142)^{f}	0.876 ± 0.034 (1.093)	1.200 ± 0.017 (1.094)^{c}
AP 100 mg/Kg	3.232 ± 0.210 (1.177)	0.040 ± 0.004 (1.249)^{f}	2.230 ± 0.171 (1.109)	16.355 ± 1.013 (1.078)f	19.096 ± 0.591 (1.118)^{d}	0.885 ± 0.045 (1.104)	1.110 ± 0.040 (1.012)
AI/AP-50 mg/Kg	2.875 ± 0.286 (1.047)	0.037 ± 0.002 (1.149)^{f}	2.511 ± 0.170 (1.248)^{f}	18.149 ± 0.980 (1.196)f	22.188 ± 2.126 (1.299)^{f}	0.885 ± 0.064 (1.104)	1.083 ± 0.026 (0.988)
AI/AP-100 mg/Kg	3.352 ± 0.395 (1.220)	0.036 ± 0.003 (1.134)^{f}	2.536 ± 0.096 (1.261)^{cf}	16.694 ± 1.594 (1.232)^{f}	22.457 ± 1.654 (1.315)^{f}	0.876 ± 0.077 (1.092)	0.990 ± 0.006 (0.902)^{cf}

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. $^{d}p \leq 0.001$, $^{e}p \leq 0.01$ and $^{f}p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 37: Modulatory influences of LEPT in kidneys tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	2.472 ± 0.175 (1.000)	0.032 ± 0.004 (1.000)	2.239 ± 0.192 (1.000)	17.615 ± 0.779 (1.000)	17.199 ± 1.856 (1.000)	1.079 ± 0.036 (1.000)	1.060 ± 0.009 (1.000)
DMBA/Croton Oil	2.236 ± 0.119 (0.904)	0.028 ± 0.001 (0.864)	2.017 ± 0.128 (0.901)	15.476 ± 0.509 (0.879)	14.270 ± 0.528 (0.830)	1.309 ± 0.043 (1.213)[c]	1.190 ± 0.034 (1.123)
AI 50 mg/Kg	2.369 ± 0.187 (0.958)	0.029 ± 0.002 (0.910)	2.185 ± 0.101 (0.976)	16.192 ± 1.314 (0.919)	16.773 ± 1.698 (0.975)	1.291 ± 0.068 (1.196)	1.168 ± 0.055 (1.102)
AI 100 mg/Kg	2.412 ± 0.072 (0.975)	0.041 ± 0.002 (1.261)[e]	2.396 ± 0.185 (1.070)	17.707 ± 0.855 (1.005)	19.728 ± 1.466 (1.147)[f]	1.319 ± 0.094 (1.222)	1.137 ± 0.058 (1.072)
AP 50 mg/Kg	2.447 ± 0.119 (0.990)	0.034 ± 0.002 (1.043)	2.327 ± 0.199 (1.039)	16.315 ± 0.208 (0.926)	17.628 ± 1.842 (1.025)	1.170 ± 0.073 (1.084)	1.092 ± 0.129 (1.030)
AP 100 mg/Kg	2.717 ± 0.104 (1.099)[f]	0.044 ± 0.003 (1.366)[ce]	2.532 ± 0.180 (1.131)	18.352 ± 0.658 (1.042)[f]	18.729 ± 1.551 (1.089)[f]	1.174 ± 0.059 (1.087)	1.041 ± 0.059 (0.982)
AI/AP-50 mg/Kg	3.253 ± 0.262 (1.316)[f]	0.038 ± 0.004 (1.165)	2.596 ± 0.161 (1.159)[f]	17.965 ± 1.433 (1.020)	22.105 ± 1.776 (1.285)[f]	1.131 ± 0.087 (1.048)	0.908 ± 0.050 (0.857)[ce]
AI/AP-100 mg/Kg	3.057 ± 0.184 (1.236)[f]	0.048 ± 0.004 (1.473)[ce]	2.903 ± 0.144 (1.297)[cf]	21.051 ± 1.056 (1.195)[e]	20.981 ± 1.415 (1.220)[f]	1.099 ± 0.045 (1.018)[f]	0.884 ± 0.037 (0.834)[ce]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 38: Modulatory influences of LEPT in forestomach tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	2.235 ± 0.201 (1.000)	0.026 ± 0.002 (1.000)	1.482 ± 0.035 (1.000)	12.807 ± 0.669 (1.000)	17.557 ± 1.589 (1.000)	0.856 ± 0.087 (1.000)	1.141 ± 0.065 (1.000)
DMBA/Croton Oil	1.837 ± 0.142 (0.822)	0.022 ± 0.000 (0.845)	1.336 ± 0.061 (0.901)	11.575 ± 0.674 (0.904)	15.601 ± 0.291 (0.889)	1.071 ± 0.047 $(1.252)^{c}$	1.416 ± 0.035 $(1.241)^{c}$
AI 50 mg/Kg	2.435 ± 0.230 (1.089)	0.023 ± 0.001 (0.892)	1.380 ± 0.064 (0.931)	12.498 ± 0.878 (0.976)	15.949 ± 1.184 (0.908)	1.026 ± 0.068 (1.199)	1.328 ± 0.089 (1.163)
AI 100 mg/Kg	2.274 ± 0.118 (1.019)	0.026 ± 0.002 (1.007)	1.607 ± 0.112 (1.084)	12.903 ± 0.397 (1.007)	18.657 ± 1.558 (1.063)	1.008 ± 0.019 (1.178)	1.242 ± 0.052 (1.088)
AP 50 mg/Kg	2.763 ± 0.289 $(1.236)^{f}$	0.027 ± 0.001 $(1.042)^{e}$	1.595 ± 0.131 (1.076)	13.361 ± 0.327 (1.043)	18.553 ± 1.403 (1.057)	0.985 ± 0.040 (1.151)	1.241 ± 0.088 (1.087)
AP 100 mg/Kg	2.700 ± 0.088 $(1.208)^{e}$	0.026 ± 0.002 $(1.027)^{f}$	1.653 ± 0.088 $(1.115)^{f}$	13.131 ± 0.378 (1.025)	18.988 ± 0.881 $(1.082)^{f}$	0.952 ± 0.038 (1.112)	1.118 ± 0.061 $(0.979)^{e}$
AI/AP-50 mg/Kg	2.815 ± 0.294 $(1.259)^{f}$	0.029 ± 0.002 $(1.121)^{f}$	1.635 ± 0.092 $(1.103)^{f}$	12.641 ± 0.713 (0.987)	18.578 ± 1.424 (1.058)	0.911 ± 0.041 (1.064)	1.100 ± 0.026 $(0.964)^{e}$
AI/AP-100 mg/Kg	3.758 ± 0.338 $(1.681)^{ce}$	0.033 ± 0.003 $(1.291)^{f}$	1.738 ± 0.050 $(1.173)^{ce}$	13.733 ± 0.355 $(1.072)^{f}$	20.321 ± 1.511 $(1.157)^{f}$	0.788 ± 0.013 $(0.920)^{e}$	1.071 ± 0.043 $(0.939)^{e}$

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. $^{d}p \leq 0.001$, $^{e}p \leq 0.01$ and $^{f}p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 39: Modulatory influences of LEPT in spleen tissue against DMBA induced and croton oil promoted skin papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	1.418 ± 0.141 (1.000)	0.029 ± 0.003 (1.000)	0.937 ± 0.030 (1.000)	8.687 ± 0.525 (1.000)	11.545 ± 1.018 (1.000)	1.106 ± 0.077 (1.000)	0.924 ± 0.050 (1.000)
DMBA/Croton Oil	1.255 ± 0.067 (0.885)	0.023 ± 0.002 (0.779)	0.798 ± 0.082 (0.852)	7.462 ± 0.531 (0.859)	10.533 ± 0.856 (0.912)	1.356 ± 0.055 (1.226)[c]	1.079 ± 0.094 (1.168)
AI 50 mg/Kg	1.269 ± 0.080 (0.895)	0.026 ± 0.002 (0.882)	0.939 ± 0.004 (1.002)	7.743 ± 0.650 (0.891)	11.642 ± 0.993 (1.008)	1.325 ± 0.068 (1.198)	1.044 ± 0.047 (1.130)
AI 100 mg/Kg	1.366 ± 0.014 (0.963)	0.032 ± 0.002 (1.105)[f]	1.039 ± 0.087 (1.110)	8.958 ± 0.673 (1.031)	12.153 ± 0.394 (1.053)	1.294 ± 0.058 (1.170)	0.962 ± 0.040 (1.042)
AP 50 mg/Kg	1.504 ± 0.054 (1.061)[f]	0.028 ± 0.003 (0.954)	1.045 ± 0.048 (1.116)	8.907 ± 0.749 (1.025)	11.903 ± 0.711 (1.031)	1.301 ± 0.073 (1.177)	0.962 ± 0.019 (1.041)
AP 100 mg/Kg	1.517 ± 0.050 (1.070)[f]	0.031 ± 0.002 (1.082)[f]	1.058 ± 0.046 (1.130)	9.234 ± 0.560 (1.063)[f]	12.568 ± 0.523 (1.089)	1.218 ± 0.086 (1.101)	0.878 ± 0.013 (0.951)
AI/AP-50 mg/Kg	1.362 ± 0.054 (0.961)	0.031 ± 0.002 (1.053)[f]	1.076 ± 0.011 (1.149)[c]	8.749 ± 0.602 (1.007)	11.474 ± 0.615 (0.994)	1.264 ± 0.115 (1.143)	0.937 ± 0.017 (1.014)
AI/AP-100 mg/Kg	1.595 ± 0.159 (1.125)	0.032 ± 0.002 (1.101)[f]	1.154 ± 0.092 (1.232)[f]	9.372 ± 0.452 (1.079)[f]	12.409 ± 0.176 (1.075)	1.105 ± 0.050 (0.999)[f]	0.804 ± 0.032 (0.870)[f]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only DMBA/croton oil treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 40: Modulatory influences of FENA in lung tissue against B(a)P induced forestomach papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD Η	CAT κ	LDH φ	NO ψ
Control	2.928 ± 0.294 (1.000)	0.036 ± 0.003 (1.000)	2.154 ± 0.091 (1.000)	15.800 ± 0.947 (1.000)	15.306 ± 1.020 (1.000)	1.066 ± 0.049 (1.000)	1.038 ± 0.062 (1.000)
Benzo(a)pyrene	2.766 ± 0.263 (0.945)	0.027 ± 0.000 (0.738)^{c}	1.943 ± 0.056 (0.902)	12.982 ± 0.466 (0.822)^{c}	12.534 ± 1.203 (0.819)^{c}	1.260 ± 0.084 (1.182)	1.227 ± 0.065 (1.183)^{c}
25 mg/kg	2.879 ± 0.196 (0.983)	0.037 ± 0.004 (1.027)^{f}	2.235 ± 0.235 (1.037)	14.093 ± 1.302 (0.892)	15.245 ± 1.041 (0.996)	1.119 ± 0.096 (1.051)	1.230 ± 0.048 (1.185)
50 mg/kg	2.950 ± 0.201 (1.008)	0.041 ± 0.004 (1.127)^{f}	2.478 ± 0.239 (1.150)	16.144 ± 1.506 (1.055)	16.127 ± 0.491 (1.054)	1.073 ± 0.112 (1.007)	1.072 ± 0.024 (1.033)
100 mg/kg	3.158 ± 0.232 (1.079)	0.039 ± 0.003 (1.081)^{f}	2.934 ± 0.225 (1.362)^{cf}	17.202 ± 1.047 (1.089)^{f}	16.491 ± 1.175 (1.077)^{f}	1.072 ± 0.039 (1.006)	0.983 ± 0.054 (0.948)^{f}

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . $^{a}p \leq 0.001$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.05$ represent significant changes against control group. $^{d}p \leq 0.001$, $^{e}p \leq 0.01$ and $^{f}p \leq 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 41: Modulatory influences of FENA in kidneys tissue against B(a)P induced forestomach papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD H	CAT κ	LDH φ	NO ψ
Control	2.399 ± 0.203 (1.000)	0.027 ± 0.002 (1.000)	2.234 ± 0.156 (1.000)	15.885 ± 1.209 (1.000)	19.233 ± 1.638 (1.000)	1.013 ± 0.088 (1.000)	1.174 ± 0.080 (1.000)
Benzo(a)pyrene	2.185 ± 0.198 (0.911)	0.020 ± 0.002 (0.747)[c]	2.085 ± 0.101 (0.933)	13.808 ± 0.922 (0.869)	16.326 ± 0.187 (0.849)	1.264 ± 0.051 (1.248)[c]	1.319 ± 0.039 (1.123)
25 mg/kg	2.573 ± 0.232 (1.073)	0.022 ± 0.001 (0.825)	2.515 ± 0.143 (1.125)	16.039 ± 1.465 (1.010)	19.888 ± 2.048 (1.034)	1.136 ± 0.047 (1.122)	1.181 ± 0.040 (1.006)
50 mg/kg	2.933 ± 0.276 (1.223)[f]	0.039 ± 0.003 (1.447)[ce]	2.517 ± 0.164 (1.127)	16.260 ± 1.631 (1.024)	21.950 ± 0.765 (1.141)[e]	1.043 ± 0.042 (1.030)[f]	1.152 ± 0.113 (0.981)
100 mg/kg	2.915 ± 0.124 (1.215)[f]	0.037 ± 0.002 (1.388)[be]	2.769 ± 0.153 (1.239)[cf]	18.766 ± 1.012 (1.181)[f]	24.890 ± 2.235 (1.294)[f]	0.944 ± 0.013 (0.932)[f]	1.036 ± 0.028 (0.882)[e]

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 42: Modulatory influences of FENA in spleen tissue against B(a)P induced forestomach papillomagenesis

Groups	GST γ	DTD δ	GSH ε	SOD η	CAT κ	LDH φ	NO ψ
Control	1.348 ± 0.094 (1.000)	0.027 ± 0.004 (1.000)	0.906 ± 0.038 (1.000)	8.547 ± 0.264 (1.000)	14.732 ± 1.378 (1.000)	1.208 ± 0.095 (1.000)	0.864 ± 0.027 (1.000)
Benzo(a)pyrene	1.070 ± 0.106 (0.794)[c]	0.022 ± 0.000 (0.827)	0.839 ± 0.036 (0.925)	7.692 ± 0.444 (0.900)	13.125 ± 0.832 (0.891)	1.664 ± 0.209 (1.377)	1.056 ± 0.041 (1.223)[c]
25 mg/kg	1.285 ± 0.106 (0.953)	0.024 ± 0.001 (0.912)	0.859 ± 0.070 (0.948)	7.930 ± 0.349 (0.928)	14.272 ± 1.166 (0.969)	1.579 ± 0.093 (1.306)[c]	0.991 ± 0.053 (1.147)
50 mg/kg	1.450 ± 0.032 (1.076)[f]	0.025 ± 0.003 (0.936)	1.075 ± 0.056 (1.185)[f]	8.402 ± 0.780 (0.983)	14.413 ± 0.587 (0.978)	1.442 ± 0.058 (1.193)	0.966 ± 0.068 (1.118)
100 mg/kg	1.444 ± 0.105 (1.072)[f]	0.027 ± 0.002 (1.017)[f]	1.104 ± 0.061 (1.217)[cf]	9.149 ± 0.726 (1.070)	15.548 ± 1.533 (1.055)	1.451 ± 0.024 (1.201)[c]	0.922 ± 0.016 (1.067)

Values are expressed as mean ± SEM of 4–6 animals. Values in parentheses represent mean fold change in the activity/level of parameter assessed in comparison to control mice. . [a]$p \leq 0.001$, [b]$p \leq 0.01$ and [c]$p \leq 0.05$ represent significant changes against control group. [d]$p \leq 0.001$, [e]$p \leq 0.01$ and [f]$p \leq 0.05$ represent significant changes against only B(a)P treated group. γ µmole CDNB-GSH conjugate formed/min/mg protein, δ µmole of DCPIP reduced/min/mg protein, ε nmole GSH/g tissue, η µmole/mg protein, κ µmole H_2O_2 consumed/min/mg protein, φ µmole of NADH oxidized/min/mg protein and ψ nmole NO/g tissue.

Table 43: ADME/Tox profile of phytochemicals present in LEPT

No.	Absorption				Distribution		Metabolism		Excretion		Toxicity					
	WS	CP	IA	SP	BBBP	CNSP	CYPS	CYPI	TC	ROCTS	Gentox.	MTD	ORAT	ORCT	HepTox	SS
1	-5.702	1.476	91.551	-2.593	0.028	-1.307	No	1A2	1.565	No	No	1.247	1.916	3.123	No	Yes
2	-6.616	1.538	94.59	-2.587	0.905	-1.02	No	1A2, C19	1.812	No	No	1.529	1.784	3.053	No	Yes
3	-6.817	1.249	90.857	-2.727	-0.099	-0.788	No	1A2, C19	1.913	No	No	1.483	1.701	0.856	No	Yes
4	-7.026	1.318	94.715	-2.099	0.753	-0.1094	No	1A2, C20	0.155	No	No	1.533	2.233	1.271	No	Yes
5	-8.125	1.357	94.67	-2.655	0.77	-0.716	3A4	No	0.594	No	No	0.822	1.971	1.036	No	No

WS: Water solubility (log mol/L), CP: Caco-2 Permeability (log Papp in 10-6 cm/s), IA: Intestinal absorption (human) (% Absorbed), SP: Skin Permeability (log Kp), BBBP : Blood-permeability brain barrier P(log BB), CNSP: CNS permeability (log PS), CYPS: CYP450 substrate, CYPI: CYP450 inhibitor, TC: Total Clearance (log ml/min/kg), ROCTS: Renal OCT2 substrate, Gentox: Genotoxicity (AMES), ORCT: Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)MTD: Maximum tolerated dose (human) (log mg/kg/day), ORAT: Oral Rat Acute Toxicity (LD50) (mol/kg), HepTox: Hepatotoxicity, SS: Skin Sensitization.

Compounds: 1. n-Heaxdecanoic acid, 2. 9-Octadecenoic acid (Z)-, methyl ester, 3. 13-Docosenamide, (Z)-, 4. Farnesyl bromide and 5. Stigmastan-3,5-diene.

6.4 Discussion and conclusion

It has been established from several experimental and epidemiological studies that regular consumption of fruits and vegetables rich in phytochemicals confers chemopreventive potentials against a large number of cancers. [305,306] Therefore, it has become increasingly important to screen the medicinal plants that have chemopreventive potentials from the plethora of traditional and indigenous knowledge and to establish them in proper scientific way. [307] In the present study, we have examined and established the chemopreventive potentials of hydroalcoholic flower extract of *Nyctanthes arbor-tristis* (FENA) and leaf extract of *Phlogacanthus thyrsiflorus* (LEPT) against DMBA induced and croton oil promoted skin papillomagenesis and Benzo(a)pyrene [B(a)P] induced forestomach papillomagenesis. The investigated doses are selected based on the LD_{50} values of the individual phytochemicals present in the extract. The treatment of mice with FENA/LEPT did not cause any apparent changes related to toxicity and mortality. The non-toxic behavior of FENA and LEPT was also deduced from the LDH activity measured in hepatic and extra hepatic organs. This decrease in the activity of LDH in most of the organs by the treated doses suggests non-toxic effect of the extract on the cellular system which might have decreased the endogenous cellular injury (as a part of normal cellular metabolism). [254,255]

The DMBA/croton oil model of skin papillomagenesis offers an excellent opportunity to monitor the chemopreventive potential at three different phases of carcinogenesis *viz.* initiation, promotion and progression. [308] Here, in this model topical application of DMBA forms DNA adduct by binding to the N^6 position of deoxyadenosine and causes A:T to T:A transversions, which can result in mutations and initiate papilloma in the skin. Repetitive topical application of croton oil promotes the tumorigenesis. [309] Oral feeding of mice with FENA/LEPT at different phase of papillomagenesis inhibit skin papilloma incidence and multiplicity as well as papilloma yield and burden across the phases. Histologically, the skin of normal mice consists of two layers: epidermis composed of stratified squamous epithelium and dermis composed of connective tissues. [310] After continuous exposure of carcinogens like DMBA/croton oil, the epidermis layer

start to display hyperkeratosis (thickening of the stratum cornea), acanthosis (thickening through hyperplasia of the stratum spinosum), papillomatosis (appearance of distinct papilloma) and infiltrate towards dermis [28. On the other hand, the connective tissues present in the dermis layers starts neovascularization/angiogenesis to nourish the upper epithelium. [311,312] This was evident from the histology of skin of DMBA/croton oil treated group. The treatment of FENA/LEPT to the mice at various phase of papillomagenesis demonstrated varying degree of improvement in the histological texture which supports the observed chemopreventive potentials. In this study, we have observed a significant increase in the rate of body weight growth along with relative weight gain of lung and kidneys when treated with FENA in pre-initiation phase. The topical application of DMBA/croton oil induced inflammation and subsequently lowered the diet intake and nutrient absorption or increased energy expenditure resulting into decrease in overall body weight growth and relative organ weight. [313]

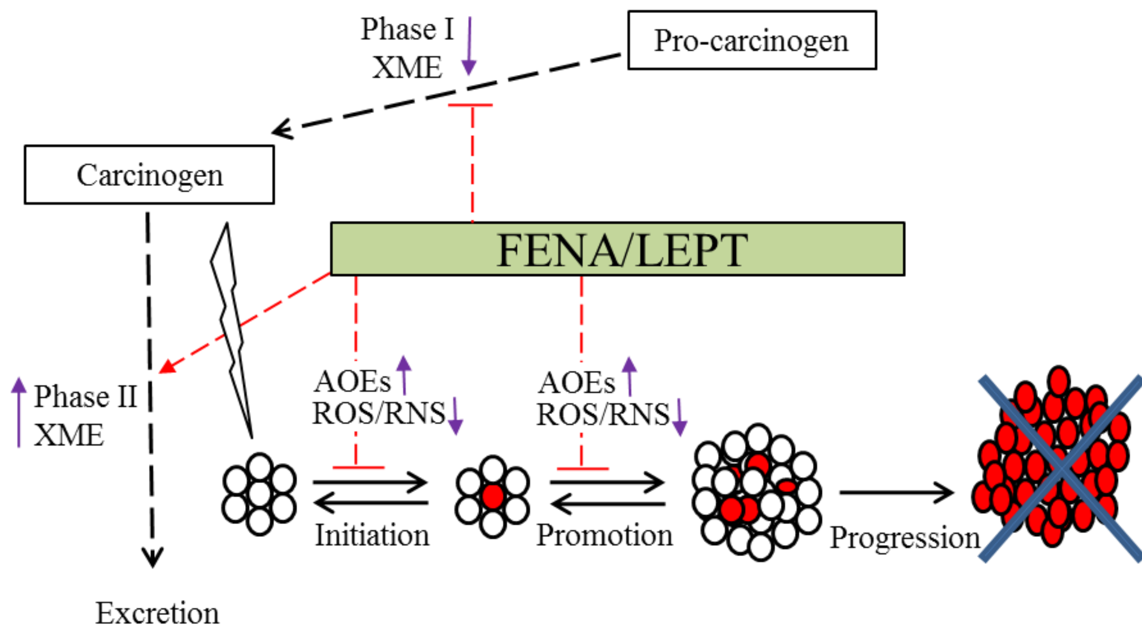


Figure 34: Proposed mode of chemopreventive potentials of FENA and LEPT

Benzo(a)pyrene, a potent pro-carcinogen of polyaromatic hydrocarbons (PAHs) type, present in the environment, generated from the combustion sources such as diesel exhaust, cigarette smoke, industrial coke production and known to initiate and promote papilloma when ingested orally. [95,314] B(a)P is metabolically activated into its active from (+)-anti-7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene [(+)-anti-BaPDE] and its covalent interaction with nucleophilic sites in DNA initiate papilloma in the forestomach. [94,315] Hence, the induction of forestomach papillomagenesis by oral administration of B(a)P offer opportunity to study the chemopreventive potential. In the present study, oral feeding of FENA or LEPT inhibited the papilloma multiplicity, yield and burden prominently in dose dependent manner. The observed chemopreventive efficacy was also supported by the histological observation of the sections of forestomach from normal and treated groups. The mice that received FENA or LEPT showed restoration of B(a)P induced histological changes in stratified squamous epithelium as well as in mucosa and submucosa. However, the papilloma related characters like hyperkeratosis, acanthosis etc. were not fully diminished in extract treated group. Treatment with FENA resulted in non-significant restoration of the hypertrophy and rate of body weight growth. Administration of B(a)P through oral gavage route significantly induced toxicological hypertrophy (increase in size of organ) of liver and forestomach and subsequently reduced the diet intake resulting into decreased body weight which might be responsible for non-significant restoration of the hypertrophy and rate of body weight growth in the present situation. [316,317] The B(a)P induced hypertrophy of liver, lungs and forestomach were significantly restored in LEPT treated group. The rate of body weight gain was also observed to be increase in LEPT treated group as compared to only B(a)P treated. However, there was no correlation between weight gain of liver, lungs, forestomach and overall body weight, which suggests that other organs may be involved in alteration of overall body weight.

There are several biochemical transformation systems in animals that either contribute to carcinogenesis or help in protecting from the insult of carcinogenesis. [318] The phase I metabolism system (cytochrome P450system) either activates xenobiotics into

active forms or prepare them for phase II metabolism and phase II systems (GST, DTD) detoxify the xenobiotic by converting into water soluble and extractable products. [94,319] FENA and LEPT are found to inhibit the activity of hepatic cytochrome P450 reductase and b5 reductase and thereby blocks the activation of pro-carcinogenic compounds into ultimate carcinogens and prevents papillomagenesis. Glutathione-*S*-transferase (GST) is a critical detoxification enzyme that conjugates the "functionalized p450 metabolites" with reduced glutathione, an endogenous ligand thereby favouring their elimination from the body. [320] DT-diaphorase metabolises the quinones and their metabolic precursors like polycyclic aromatic hydrocarbon, benzene, etc. into stable hydrocarbon by two electron oxido-reduction, preventing conversion into reactive intermediate and thereby provide protection to the animal system. [47,48] It is evident that induction of GST and DTD confers protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals by decreasing their bioavailability. [49-52] In the present study, the inducibility of GST and DTD by FENA/LEPT in majority of the organs, provide insight into the chemopreventive efficacy. The inducibility of both the phase II enzymes and ability to reduce phase I enzymes indicates the 'bifunctional' efficacy of FENA and LEPT. [307]

The critical balance between reactive species and antioxidant status of an organism is also a determinant of pathogenesis. Reactive oxygen species (ROS), e.g. superoxide anion radical (O_2^{*-}), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^*OH$) etc. are generated from the metabolism of molecular oxygen and normally exist in balance with cellular antioxidants. [321,322] In contrast to ROS, reactive nitrogen species (RNS) such as NO_2^-, $ONOO^-$, N_2O_3, HNO_2 etc., are generated from the intercellular messenger Nitric Oxide (NO). [323,324] The disruption of this critical balance between ROS/RNS and antioxidants leads to oxidative stress and causes damage to the biomolecules such as DNA, RNA, protein, lipids etc. and finally results in initiation of cancer. [321,322] Cellular antioxidant enzyme system scavenges and/or neutralizes free radicals and/or harmful effects of free radicals and thereby provides protection to the organism. [325,326] Reduced glutathione (GSH), a cellular non-enzymatic antioxidant plays role in maintaining redox balance by directly or indirectly interacting with reactive

species, and is also involved in detoxification of electrophiles either via direct interactions or via enzyme-catalyzed conjugation in phase II metabolism. [55] The ability of phytochemicals to disrupt the link between elevated ROS/RNS and depleted GSH can be used as antioxidant therapy in chemoprevention of cancer. [56] The regulation of glutathione homeostasis is maintained by enzyme glutathione reductase (GR) which reduces the oxidized glutathione (GSSG) to reduced glutathione (GSH) at the expense of NADPH. [57,58] The present investigation reveals that the FENA and LEPT increases the activity of hepatic GR and GSH level in most of the organs and thereby maintains the redox homeostasis to prevent tumorigenesis. Superoxide dismutase (SOD) is known to detoxify the superoxide radicals generated endogenously or exogenously, by converting them into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2^{*-}). [58] When this cellular hydrogen peroxide exists in low concentration, glutathione peroxidase (GPx) detoxifies it into water. However, upon GPx pathway reaching saturation with the substrate, CAT comes into play and detoxifies the excess hydrogen peroxide from the cellular system. [59] In the present treatment condition, the extracts were found to induce the activities of hepatic GPx and SOD as well as catalase in most of the tissues suggesting probable protective function against pro-oxidant-mediated injury and subsequently prevents the initiation of cancer. Malondialdehyde (MDA) and 4-hydroxylnonenenal (HNE) are the major aldehyde products of lipid peroxidation and are known to be mutagenic and react with DNA to form adducts/damage. [327] Therefore, inhibition of lipid peroxidation is an important attribute of chemoprevention and in our study, FENA and LEPT was found to be effective in decreasing MDA formation, both in hepatic and papilloma bearing tissues.

The FENA and LEPT contains several phytochemicals as ascertained from GC-MS analysis that may contribute to the observed chemoprevention. It contains oleic acid, which is known to reduce the risk of breast cancer and possess anticancer activity in various cancer models through activation of several intracellular pathways. [328-331] The extract also contains phytol which is known to exert antitumor activity through involvement of caspase-9/3 activation and inhibition of epithelial mesenchymal

transition (EMT) in hepatocellular carcinoma cells. [332,333] The mixture of phytochemicals present in crude extract could have conferred the observed chemopreventive effect through the modulation of several biochemical and molecular pathways related to tumorigenesis [Figure 35].

The findings of present investigation clearly demonstrate that FENA and LEPT inhibit DMBA induced and croton oil promoted skin papillomagenesis and B(a)P induced forestomach papillomagenesis in *Swiss albino* mice through differential modulation of enzymes of xenobiotic metabolism, antioxidants and reactive species in hepatic, extrahepatic as well as in papilloma bearing tissues. Our findings further leads to scientific validation of the traditional use of flower of *Nyctanthes arbor-tristis* and leaf extract of *Phlogacanthus thyrsiflorus* and suggest that these two plants may be utilized for the chemoprevention of cancer.