CHAPTER - 8

PUBLICATION
JOURNAL OF
APPLIED BIOSCIENCES &
BIOTECHNOLOGY
Effect of Eclipta alba L. on Hepatic Detoxification Enzyme-
Glutathione S transferase and Liver Somatic Index in 
Female Swiss Albino Mice

Sabera Sultana Rehman and R Bharali
Department of Biotechnology, Gauhati University, Guwahati-781014, Assam.
E mail : rbharali@rediffmail.com

ABSTRACT
In the present investigation, the effect of Eclipta alba extract on glutathione S transferase, 
Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase 
(SGPT) and the liver somatic index was evaluated with a modulator dose of 125mg/kg body 
weight (low dose) and 250mg/kg body weight (high dose) through oral gavage for a period of 
14 days. The animals selected for the experiment were female Swiss Albino mice of 6-8 
weeks old. There was no significant alteration in the levels of SGOT and SGPT in comparison 
to the control group. Significant elevation in the specific activity of hepatic glutathione S-
transferase (GST) was observed in comparison to the control. Butylated hydroxyanisole (BHA), 
which served as a positive control, was fed in diet at a dose level of 0.75% daily for duration 
of 14 days and exhibited a significant augmentation in the specific activity of GST. The 
findings of the present study postulated that Eclipta alba had no adverse effect on the mice at 
the selected dose levels and it has immense potential to be used as a medicinal plant.

Keywords : Eclipta alba, SGOT, SGPT, Glutathione S transferase.

Introduction
The human body is exposed to a wide array of 
exenobiotics in one's lifetime, from food components 
to environmental toxins to pharmaceuticals and has 
developed complex enzymatic mechanism to detoxify 
these substances1. Liver is the main organ responsible 
for drug metabolism and appears to be a sensitive target 
site for substances modulating biotransformation2. 
Amongst the liver enzymes, GST plays an important 
role against cancer by detoxifying xenobiotics with 
mutagenic potential3. Glutathione S transferase 
conjugate drugs, poisons and other compounds with 
reduced glutathione to facilitate dissolution in the 
aqueous cellular and extracellular media, and from 
there, out of the body4. Modulation of these enzymes 
by chemopreventive agents is an important part of their 
mechanism of action5,6. The liver enzymes, glutamate 
oxaloacetate transaminase and glutamate pyruvate 
transaminase are known to boost vastly when the body 
comes in contact with xenobiotics, drugs, poisons 
etc thereby indicating the extent of liver damage7. Herbs 
have recently attracted much attention as health 
beneficial foods and plant extracts are being increasingly utilized to treat a wide variety of clinical 
diseases including liver disease, ischemia and cancer8, 
9. Eclipta alba, a perennial herb belonging to the family 
Compositae is widely used in traditional Chinese herbal 
medicine as a rejuvenative and liver tonic10. The plant 
is found mostly in wet and moist places in the lowlands 
and paddy fields of Japan, China, Korea and Australia11. 
In India it is found throughout the region, upto 2000 m 
on the hills12. The plant is used as an astringent, 
deblocking, depurative, emetic, ophthalmic, purgative 
and tonic13. The herb contains wedelolactone and 
desmethyl wedelolactone as the active principles14, 
besides containing the flavonoids apigenin, b-amyrin, 
stigmasterol and luteolin15. The purpose of the present 
investigation is to find out the medicinal efficacy of 
Eclipta alba extract on the activity of Glutathione S 
transferase and the liver somatic index as well as to 
determine the SGOT and SGPT levels in Swiss Albino 
mice.
Effect of Eclipta alba L. on Hepatic

Materials and Methods

Chemicals: Reduced glutathione, 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma Chemicals Co. (St. Louis, USA). Bovine serum albumin, Folin ciocalteau reagent etc. were obtained from local firms (India) and are of highest purity grade.

Test material: The herb, Eclipta alba was collected locally from various parts of Guwahati, Assam, India and proper identification was done by a plant taxonomist from the Department of Botany, Gauhati University, Guwahati, Assam. The plant was washed and shade dried without direct exposure to sunlight. It was then grounded and 50g of the material thus obtained was subjected to soxhlet extraction using 300 ml of hydro-alcoholic solvent (80% solvent: 20 % distilled water). The process was repeated 3 times with fresh material of the same amount. The alcohol was allowed to evaporate and then the residue obtained was stored at 4°C.

Selection of modulator dose: Prior to the administration of the extract in the experiment, a pilot study was carried out in order to check the safety of the test dose by the method reported by Das et al. (2005)16. A dose of 250 mg / kg, per oral and 125 mg/kg, per oral of the extract was selected arbitrarily for testing its safety for a period of 14 days which is also the duration followed for the experiment. Two groups of female mice containing six mice in each group were treated with a daily dose of 250mg/kg, per oral and 125mg/kg, per oral for 14 days. Mortality along with other symptoms like significant behavioral changes was recorded.

Animals: Random bred 7-8 weeks old female Swiss Albino mice were used to carry out the experiments. The animals were obtained from the animal house of Zoology Department, Gauhati University and housed under normal conditions having natural photoperiod. They were provided with standard pellet diet and tap water adlibitum, under hygienic conditions

Experimental design: Modulatory influence of Eclipta alba on drug metabolizing enzyme in mice was evaluated as per the method reported by Bharali et al (2003)17 and Kumar et al. (2006)18. The animals were randomly assorted into the following groups.

Group I (n =10): This group of animals received a normal diet of standard pellets daily for 14 days and served as a negative control.

Group II (n =10): This group of animals received a normal diet and treated daily with 125 mg / kg body weight of Eclipta alba extract in distilled water through oral gavage for 14 days.

Group III (n =10): This group of animals received a normal diet and treated daily with 250 mg / kg body weight of Eclipta alba extract in distilled water, through oral gavage for 14 days.

Group IV (n =10): This group of animals received a normal diet containing 0.75 % BHA for 14 days and served as a positive control.

Body weights of the mice were recorded initially, at weekly intervals and at the end of the experiment. Diet was withheld from the animals on the night prior to the day of termination of the experiment.

Preparation of homogenate for biochemical study: The animals were sacrificed by cervical dislocation and the gall bladder was carefully removed. Then the entire liver was perfused immediately with 0.9% ice-cold NaCl solution and thereafter carefully dissected out and rinsed in chilled 0.15 M Tris KCl buffer. The homogenate was prepared by the method of Fry and Bridges (1975)19. A portion of the liver was weighed quickly and homogenized in ice cold 0.15M Tris – KCl buffer (pH-7.4) to yield 10 % (w/v) homogenate at 4°C. The homogenate was then centrifuged at 10,000g for 20 minutes at 4°C. The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, were used for the assay of GST and protein.

Assay methods

Glutathione S transferase: The cytosolic GST activity was determined spectrophotometrically at 37°C by the method of Habig et al. (1974)20. The reaction mixture (1ml) contained 33411 of lOOmM phosphate buffer (pH-6.5), 33 11 of 30mM CDNB (in 95% ethanol), 33 11 of 30mM of reduced glutathione and 59011 distilled water. After preincubating the reaction mixture for two minutes, the reaction was started by adding 10 lI of diluted cytosol and then time scanned for 3 minutes at 340 nm using a Cintra-5 UV-VIS double beam spectrophotometer of GBC, Australia make.
Biochemical estimations: Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were determined following the method described in the kit\(^21\). Activities of these two enzymes were expressed as U/L. Protein was estimated by following the method of Lowry et al (1951)\(^22\) using Bovine Serum Albumin (BSA) as standard at 750nm.

Statistical analysis: In the enzymatic studies, data from different groups were analyzed and expressed as mean ± S.D. The difference in data from different groups were statistically analyzed by student's t test and the difference between the values in the groups were considered significant at 1% (p<0.01) and 5% significance level (p<0.05).\(^23\)

Results

There was no significant alteration in the levels of SGOT and SGPT in the selected dose levels in contrast to the control. As such there were no unpleasant affects on the animals at the selected dose levels of 125mg/kg body weight and 250mg/kg body weight for 14 days. However there was a significant augmentation in the levels of both SGOT and SGPT in the positive control groups (BHA treated) in contrast to the negative control group. (Table 2).

The liver somatic index (the ratio of liver weight to the final body weight) and the cytosolic protein content remained comparable to the control thereby suggesting no unfavourable outcome of the modulator on the general body metabolism of the mice. (Table 1)

The specific activity of glutathione S transferase was significantly enhanced at P<0.05 for both low and high doses of modulator treatment in relation to the control. In the BHA treated group also the specific activity was augmented in comparison to the control as can be seen in table 2.

Table 1: Modulatory influence of *Eclipta alba* and Butylated Hydroxy Anisole (BHA) on weight gain profiles and hepatic protein level in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration of treatment days</th>
<th>Body weight (g)</th>
<th>Liver weight x 100/final body weight</th>
<th>Protein (Cytosol) mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Control (only vehicle distilled water)</td>
<td>14</td>
<td>20.1±0.17</td>
<td>21.2±0.22</td>
<td>5.53±0.13</td>
</tr>
<tr>
<td>II</td>
<td><em>E. alba</em> (125mg / Kg body weight in distilled water)</td>
<td>14</td>
<td>20.2±0.20</td>
<td>21.0±0.12</td>
<td>5.59±0.10</td>
</tr>
<tr>
<td>III</td>
<td><em>E. alba</em> (250mg/ Kg body weight in distilled water)</td>
<td>14</td>
<td>20.1±0.16</td>
<td>21.0±0.09</td>
<td>5.78±0.17(^b)</td>
</tr>
<tr>
<td>IV</td>
<td>BHA (0.75% in diet; positive control)</td>
<td>14</td>
<td>20.1±0.14</td>
<td>21.0±0.05</td>
<td>5.82±0.90(^b)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.

'\(^b\)' represents significant changes against control at P < 0.05.
Table 2: Modulatory influence of Eclipta alba and BHA on mouse hepatic GST levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Duration of treatment days</th>
<th>GST (μ mole of CDNB-GSH conjugate formed/min/mg protein)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (only vehicle distilled water)</td>
<td>14</td>
<td>3.14±0.22</td>
<td>71.37±8.08</td>
<td>75.56±11.81</td>
</tr>
<tr>
<td>II</td>
<td>E. alba (125mg/Kg body weight in distilled water)</td>
<td>14</td>
<td>3.29±0.04b</td>
<td>71.55±12.28</td>
<td>76.09±11.20</td>
</tr>
<tr>
<td>III</td>
<td>E. alba (250mg/Kg body weight in distilled water)</td>
<td>14</td>
<td>3.33±0.04b</td>
<td>71.54±7.22</td>
<td>75.73±11.95</td>
</tr>
<tr>
<td>IV</td>
<td>BHA (0.75% in diet; positive control)</td>
<td>14</td>
<td>8.81±0.09b</td>
<td>99.12±9.33b</td>
<td>89.17±11.88b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D of 10 animals. 'b' represents significant changes against control at P < 0.05.

Abbreviations: BHA = Butylated hydroxyanisole, GST = Glutathione S-transferase, SGOT = Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase, CDNB = 1-chloro-2,4-dinitrobenzene, GSH = Reduced glutathione.

Discussion

In India, *Eclipta alba* is widely distributed almost throughout the region in wet and moist places, in lowlands and paddy fields, yet not much work is done to evaluate its medicinal properties. The plant has found wide application in the traditional Chinese herbal medicine as a rejuvenative and liver tonic. In Unani medicine, the juice of the plant is used for washing wounds and soft chancre and applied locally on skin infections, allergic urticaria, inflammation and over swellings. In China, tender leaves and young shoots of the herb are cooked and used as vegetables. So the present study has been undertaken to evaluate the medicinal efficacy of the plant extract in the detoxification process in the liver.
As the herb has much potential as a medicinal plant, so the toxicity study was conducted to eliminate the possibility of any adverse effects of the plant extract. Estimating the activities of serum GOT and GPT, which are enzymes originally present in higher concentration in the cytoplasm can make assessment of the liver function. When there is histopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. Lack of alteration in the SGOT and SGPT levels in the treated groups in comparison to the control is indicative of the lack of any sort of histopathy or toxicity of the plant extract at the selected dose levels of 125 mg/kg body weight and 250 mg/kg body weight. Saxena et al. 1993 have also exhibited the hepatoprotective activity of Eclipta alba extract on subcellular levels in rats.

Several studies have reported that phytochemicals having chemopreventive properties bring about an elevation in the GST level. GST forms an important component of phase II metabolism and catalyses the conjugation of electrophilic xenobiotics with GSH, thereby eliminating them from the system. They are present in very high amount in the liver cytosol and in lower amounts in other tissues. A large number of xenobiotics as well as toxic metabolites of haem are converted to less toxic compounds by glutathione S transferase by interacting with the reactive intermediates resulting from the biotransformation of drugs by covalent bonding. In the present study, it was found that the specific activity of glutathione S transferase was significantly enhanced at P<0.05 for both low and high doses of modulator treatment in relation to the control. Thus the elevated levels of GST by the extract might have improved the conjugation process thereby playing a critical role in detoxification/chemoprevention. The augmentation in the GST level might have taken place due the presence of the two coumestans- wedelolactone and desmethylwedelolactone, which are the main active principles and which were also reported to have a significant stimulatory effect on liver regeneration. Bharali et al. (2003) have also reported such elevation of GST levels by the hydroalcoholic extract of Moringa oleifera and thus reported the chemomodulatory efficacy of the plant extract.

The significant increase in the liver somatic index in comparison to the control group also suggest that there is no adverse effect of the plant extract on the general metabolism and health of the animals. Thus from the present investigation it can be postulated that Eclipta alba, which is already popularly used as a home remedy for numerous purposes such as minor skin burns or cuts, wounds, insect bites, stings etc. is an edible medicinal plant with a lot of promise. This work demands additional study and further studies on biotransformation and the antioxidant enzymes on the liver are in progress.

Reference


INHIBITORY EFFECT OF THE MEDICINAL PLANT, ECLIPTA ALBA LINN. ON SKIN CARCINOGENESIS IN SWISS ALBINO MICE

Sabera Sultana Rehman and *R Bharali
Department of Biotechnology, Gauhati University, Guwahati-781014, Assam, India

ABSTRACT

Chemoprevention using readily accessible natural substances from vegetables, fruits, herbs and spices is presently considered as one of the most noteworthy strategies for cancer prevention. The herb, Eclipta alba Linn (Family: Compositae) found commonly in moist places all over India is traditionally used as a tonic and deobstruent in hepatic and spleen enlargements. It has also got anti-inflammatory effect and may be applied to insect bites, stings, swellings and other skin diseases. The present investigation was undertaken to explore the anti tumor promoting activity of Eclipta alba on two stage skin carcinogenesis, induced by a single topical application of 7, 12 Dimethyl benz (a) anthracene (50µg/ 50µl of acetone) and two weeks later, promoted by repeated application of croton oil (1% in acetone for 3 times a week) till the end of the experiment (15 weeks). Topical application of the hydro alcoholic extract of the herb, Eclipta alba for 15 weeks at the pre, peri and post initiational stages on the shaven backs of Swiss albino mice was found to be effective in decreasing the tumor incidence (90, 77.77 and 66.6% respectively) in comparison to the control (100%). The cumulative number of papillomas, tumor yield and tumor burden were also found to be reduced significantly in E. alba treated mice. The histo-pathology of the affected skin tissue also indicated a significant reduction in the tumor size and slow growth of the tumors in the treated groups in comparison to the control. The results thus suggest a possible chemopreventive property of E. alba against DMBA induced skin papillomagenesis.

Keywords: DMBA, papillomagenesis, Eclipta alba, histopathology

INTRODUCTION

Cancer chemoprevention is a concept defined as the prevention of cancer by the administration of natural or synthesized pure chemicals, or by daily foods enriched with cancer preventive components. Particularly, food phytochemical could be important for cancer prevention. In fact, a great number of epidemiological studies of the relationship between food and cancer, together with the research in the experimental animal models, have demonstrated that daily ingestion of some vegetables and fruits could undoubtedly contribute to cancer prevention (Murakami et al., 1994).

The medicinal herb Eclipta alba belonging to the family Asteraceae is found throughout India up to 2000 meters on the hills. The plant is used as tonic, deobstruent in hepatic and spleen enlargements (Kirtikar and Basu, 1981: Kanjilal, 1997). It is also used as a detoxifying deobstruent and antiseptic herb in vitiated blood, anaemia, splenic and liver enlargements, catarrhal jaundice, hyperacidity, gastritis and dysentery (Kumar, 2002. Khare, 2004) In Ayurvedic and Unani medicine, the juice of the plant is used for washing wounds and soft chancre and applied locally on skin infections, allergic urticaria, influenza and over swellings (Khare, 2004) The plant does not show any signs of toxicity and the minimum lethal dose was greater than 2 0 g/ Kg when given orally and intraperitonially in mice. The drug is traditionally considered safe (Indian Herbal Pharmacopoeia, 2002). The plant has a reputation as an anti ageing agent in Ayurveda It is used externally for inflammation, minor cuts and burns and the fresh leaf juice is considered very effective in stopping bleeding (Sharma, 2003).

MATERIALS AND METHODS

Animals
Random bred 7-8 weeks old Female Swiss Albino mites were used to carry out the experiments. Permission was obtained from the Institutional Animal Committee of Gauhati University to pursue the experiment. The animals were obtained from the animal house of Zoology Department, Gauhati University and housed under normal conditions having natural photoperiod. They were provided with standard pellet diet and tap water ad libitum, under hygienic conditions. Three days before the onset of the experiment, the hair on the interscapular region of the mice were clipped and the resting phase of the hair growth cycle was observed. Only the mice showing no hair growth were taken for the experiment. Body weights of the mice were recorded on a weekly basis to keep a constant vigil on the health of the animals

Chemicals

Chemicals for papillomagenesis
The carcinogen, 7,12-dimethylbenz (a) anthracene (DMBA), and croton oil were procured from Sigma Chemicals Co., St Louis, USA. DMBA was dissolved at
Table 1. Chemopreventive action of *Eclipta alba* extract on DMBA induced skin papillomagenesis in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g) (Mean±S.E)</th>
<th>Cumulative number of papillomas</th>
<th>Tumor incidence (%)</th>
<th>Tumor yield</th>
<th>Tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>12.2±1.23</td>
<td>24.4±1.26</td>
<td>125</td>
<td>100</td>
<td>12.5*</td>
</tr>
<tr>
<td>(N = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>14.7±0.95</td>
<td>26.2±3.19</td>
<td>50</td>
<td>90</td>
<td>5*</td>
</tr>
<tr>
<td>(N = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>15±0.66</td>
<td>17.6±2.96</td>
<td>44</td>
<td>77.77</td>
<td>4.4*</td>
</tr>
<tr>
<td>(N = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>13.5±1.4</td>
<td>27.8±1.9</td>
<td>26</td>
<td>66.66</td>
<td>2.6*</td>
</tr>
<tr>
<td>(N = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N in parenthesis indicates the number of mice used in respective groups. * Indicates the significance level among different groups at p < 0.05.

A concentration of 50 μg in 50 μl acetone. Croton oil was mixed in acetone to give a solution of 1%.

Chemicals for histopathology
Alcohol (absolute, 90%, 70%, 50%, & 30%), xylene, haematoxylin, eosin, paraffin, 10% formalin, Mayer’s albumen and DPX.

Preparation of the Eclipta Alba Extract
The herb, *Eclipta alba* was collected locally from various parts of Guwahati, Assam, India after proper identification by a competent botanist from the Department of Botany, Gauhati University, Guwahati, Assam. The plant was washed and dried in shade without direct exposure to sunrays. It was then grounded and 50 gram of the material thus obtained was subjected to soxhlet extraction using 300 ml of hydro-alcoholic solvent (80% solvent: 20% distilled water). The process was repeated 3 times with fresh material of the same amount. The alcohol was allowed to evaporate and then the residue obtained was stored at 4°C. The required dose for treatment was prepared by diluting the residue in acetone at a dose level of 5-mg/Kg body weight/day in 100 μl acetone for 14 days (7 days before and 7 days after the application of a single dose of DMBA). Croton oil was applied as in Group-I and the experiment was continued for 15 weeks.

Preparation of Skin for Histology
Affected skin and skin with papillomas were fixed in 10% formalin and dehydrated with graded alcohols starting from 30% alcohol to absolute alcohol. Then the tissues were embedded in paraffin after clearing in xylene. Serial microtome sections (4μ) were stained with H and E. (Kehar and Wahi, 1967).

Experimental Design
A total of 40 animals were taken for the experiment and divided equally into 4 groups. The experiment was conducted for 15 weeks.

Group-I
A single dose of 50 μg of DMBA in 50 μl acetone was applied topically over the shaven area of the skin of mice. Two weeks later, croton oil (100 μl of 1% croton oil in acetone) was applied three times per week until the end of the experiment.

Group-II
Animals received a topical treatment of an ethanolic extract of the herb, *Eclipta alba* (5mg/Kg body weight/day) in 100 μl acetone for 14 days (7 days before and 7 days after the application of a single dose of DMBA). Croton oil was applied as in Group-I and the experiment was continued for 15 weeks.

Group-III
Animals received a topical treatment of *Eclipta alba* extract, starting from the time of croton oil application and continued till the end of the experiment i.e., 15 weeks. A single dose of DMBA was given as in Group-I.

Group-IV
Animals were treated topically with *E. alba* extract (5mg/Kg body weight/day) throughout the experimental period, i.e., before and after DMBA application and also at the promotional stage. Croton oil was given as in Group-I and the experiment was carried out for 15 weeks.

Morphological Observations of Papilloma Development
Body weight and papillomas appearing on the shaven area of the interscapular region of the skin of mice were recorded at weekly intervals. Only those papillomas that persisted for two weeks or more and were greater than or equal to 1 mm were considered for final evaluation of the data. Based on the following observations, the values of percent inhibition of tumor multiplicity, tumor incidence, tumor yield, tumor burden and cumulative number of papillomas were obtained and compared in all the four groups.
Observation period (weeks)

Fig. 1. Effect of Eclipta alba on cumulative number of papillomas in the treated groups of mice (group-II, III & IV) in contrast to the control (group I).

Observation period (weeks)

Fig. 2. Effect of Eclipta alba on the average number of papillomas per mouse (tumor yield) in the treated mice (group II, III & IV) in contrast to control (group I).

STATISTICAL ANALYSIS

The difference in the incidence of tumors among different groups were evaluated by student’s t test and considered significant at 5% significance level (p<0.05).

RESULTS

Effect of E alba on DMBA Induced Croton Oil Promoted Tumor Incidence, Cumulative Number, Tumor Yield and Tumor Burden of Papillomas

The findings of the present study have been depicted in table 1 and figures 1-4. The administration of the hydro alcoholic extract of Eclipta alba did not affect the body weight of the animals during the experimental period. Papillomas started appearing on the shaven interscapular region of the mice from 6-12 weeks during exposure to the initiator and promoter. The percent inhibition of tumor multiplicity reduced significantly in all the experimental groups in comparison to the control.

In the control group (group I), skin papillomas appeared in all the animals (100% tumor incidence). The cumulative number of papillomas, tumor yield and tumor burden were recorded as 125, 12.5 and 12.5 respectively (Fig. 1).
significant in comparison to the control (Group 1) at 5% probability level.

**Effect of *E alba** on the Histopathology of the Skin after Treatment with DMBA (Inducer), Croton Oil (Promoter) and *E alba* Extract (Modulator)**

The animals were sacrificed at the end of the experiment (after 15 weeks of treatment) and a section of skin from the interscapular region of the mice was taken for histopathological studies. The stains used were Haematoxylin and eosin.

In the Control animals, normal cellular structure of the skin was observed i.e. the skin consists of two major layers - the epidermis and the dermis. The epidermis consists of stratified squamous epithelium. The outermost region consists of many layers of dead usually flattened squamous cells which forms a protective covering or stratum corneum on the skin surface. The deepest layer of cells in the epidermis is called the stratum germinativum or malpighian layer that consists of a single row of living columnar cells and is separated from the underlying dermis by a basement membrane (Reith and Ross, 1977; Kotpal, 1995).

The dermis or corneum is the inner layer of skin and is composed of fibrous connective tissue and contains nerve endings, blood vessels and lymphatic vessels. Pigment cells or melanocytes are mostly located in the dermis (Kotpal, 1995) (Fig. A).

In group I animals, presence of keratinized tissue along with the papillomas was observed. The skin epithelium showed multiple papillomas characterized by the development of finger like projections protruding over the surface. Each papilloma was consisted of hyperplastic stratified squamous epithelial cells with central connective tissue core along with a large number of newly formed blood vessels. The outer lining appears to be covered by keratinized tissue (Fig. B).

In group II animals, the tumor mass appears to project out from the surface. The stratum corium of the skin was almost sloughing out. The stratum germinativum layer showed proliferative changes with large number of mitotic bodies along with large nucleoli. The cells were hyperchromic in character and extended into the dermal layer. The proliferated cells developed a mass of solid sheets, occasionally with bizarre character. Few cells showed vaculation (Fig. C).

In group III animals, the papillary outgrowths were found to be much smaller then the group I. The skin epithelium showed hyperplastic papilleric outgrowths of stratified squamous epithelium along with development of cell
nests structure of keratinized tissue. The epithelial lining showed excessive presence of melanin pigments, which was outwardly covered by degenerated keratinized tissue. Core tissue is not that distinct (Fig. D).

In group IV animals, the stratified squamous epithelium focally showed papillary projection with or without presence of core tissue. The granular layer showed large haematoxylin positive granules in the cytoplasm. The germinativum layer showed bizarre arrangement with mitotic bodies. At places the hyperplastic cells showed focal penetration into the dermis. Only remnants of cornified layer could be seen (Fig. E).

**DISCUSSION**

The skin carcinogenesis model in experimental animals has been found to be very useful system for investigating the influence of dietary chemopreventors both mechanistically and operationally (Morse and Stoner, 1993). A number of naturally occurring as well as synthetic substances have been shown to cause inhibition of chemical carcinogenesis either by preventing the formation of active carcinogens from their precursors, by preventing carcinogenic compounds by reaching reacting with critical, target sites in cells or by inhibiting/ suppressing the expression of neoplasia in cells which have already reacted with carcinogens (Wattenberg, 1983, 1985). The present study demonstrates the chemopreventive potential of *Eclipta alba* on DMBA induced skin papillomagenesis in Swiss Albino mice. Berenblum and Shubik (1947) has suggested that one sub minimal dose of carcinogen initiates the process of carcinogenesis and the treatment with croton oil promotes them to visible tumor stage. The current study also exhibited the same with 100% tumor incidence in the control group. But the administration of the hydroalcoholic extract of *Eclipta alba* at pre, peri and post initialisation phases showed a significant reduction in tumor incidence, tumor yield, tumor burden and cumulative number of papillomas. The histo-pathology of the skin showed visible reduction in the size of the tumors in comparison to the control (Fig. A-E). The treated groups also have not exhibited any distinct core tissues, thereby ruling out the possibility of newly formed blood vessels. This is perhaps due to the presence of phytoestrogens like flavonoids and coumestans present in the herb, which are considered to have an inhibitory role during the initialisation and promotional phases of cancer development (Messina and Barnes, 1991; Messina et al., 1994). Several natural and dietary compounds from vegetables, fruits, herbs and spices are being considered for the primary and secondary prevention of cancer (Mishra et al., 2003). One such compound is the phytoestrogen, which is a naturally derived compound found in plants. Two phytoestrogens (coumestans), wedelolactone and desmethyl-wedelolactone were isolated as the main active principles present in *E. alba* (Saxena et al., 1993). Both constituents showed anti hepatotoxic activity in assays using liver enzyme induced cytotoxicity in cultured rat hepatocytes. These constituents also showed a significant stimulatory effect on liver regeneration (Wagner et al., 1986). Evidences suggest that *E. alba* exerts its protective action through a reduction in GSH depletion (Wagner et al., 1986; Saxena et al., 1993). Besides the herb also contains the flavonoids, apigenin and luteolin as minor constituents in addition to the active principles (Indian Medicinal Plants, 1994; Indian Herbal Pharmacopoeia, 2002). Studies have shown that apigenin acts as proteasome inhibitor and apoptotic inducer in human leukemia cells (Chen et al., 2005). Studies have also have revealed that apigenin induce cell cycle arrest in activated microglia (Elisis et al., 2005). Researches have also shown that apigenin can inhibit pancreatic cell proliferation through G2/M cell cycle arrest (Ujiki et al., 2006) and the expression of vascular endothelial growth factor and angiogenesis in human lung cancer cells (Liu et al., 2005) which may be one of the many reasons that accounted for the lack of distinct core tissues and reduction in tumor size as seen in the histopathological slides in the plant extract treated groups (Fig. C, D, E) in comparison to the DMBA and croton oil treated group (Fig. B). Studies have also revealed that luteolin is effective in the protection of human single cell DNA from oxidative attack which indicates that *E. alba*, which has luteolin as one of its constituents may have a preventive or curative effect on the oxidative stress caused by free radicals which is responsible for a wide variety of clinical disorders including cancers (Horvathova et al., 2004). The use of the herb as a healing and restorative agent against skin diseases, inflammations, wounds and ulcers have led to the supposition that it might have either acted as an anti-inflammatory agent or inhibited the epidermal ornithine decarboxylase, a rate limiting enzyme in the biosynthesis of polyamines which appear to be a pre requisite for cell proliferation, differentiation and neoplastic transformation (Katiyar et al., 1996). Thus the reduction in tumorigenesis in groups II (where animals were treated with the modulator 7 days before and 7 days after the application of a single dose of DMBA), III (where the modulator treatment was started from the time of croton oil application and continued till the end of the experiment) and IV (where the modulator treatment was continued throughout the experiment) may be due to the inhibition of epidermal ornithine decarboxylase. Similar reduction of tumorigenesis through the inhibition of epidermal ornithine decarboxylase in mice by *Emblica officinalis* (amla), a popular fruit in India have also been reported earlier (Mou et al., 1988; Sancheti et al., 2005). Similar inhibition of epidermal ornithine decarboxylase in male Wistar rats by *Butea monosperma*, a medicinal plant have been reported by Sehrawat and Sultana (2006) and by Sancheti and Goyal (2006) in mice using the extract of...
Rosemarinus officinalis, which is an evergreen shrub having medicinal properties. Further it is suggested that aryl hydrocarbon hydroxylase, a cytochrome dependent carcinogen metabolizing enzyme present in the skin appears to play an important role in the activation of polycyclic aromatic hydrocarbons into reactive moieties that can bind to DNA and that may directly induce cancer (Bickers and Kappas, 1978). So there is a possibility that E. alba might have an inhibitory influence on the aryl hydrocarbon hydroxylase enzyme system, thereby reducing tumorigenesis in the treated animals. Similar inhibition of aryl hydrocarbon hydroxylase enzyme system in rats using garlic oil have been reported by Siddiqui and Pawar (1984) and Sadhana et al. (1988) using garlic oil in mice. The significant reduction in the tumor incidence, tumor burden, tumor yield and also in the cumulative number of papillomas in the treated animals (groups II, III and IV) may be attributed to the individual or shared effects of one of the constituents of the herb, Eclipta alba.

Eclipta alba is already popularly used as a home remedy for numerous purposes such as minor skin burns or cuts, wounds, insect bites, stings etc (Indian Medicinal Plants, 1994). The present study suggests that E. alba should be explored further for cancer chemopreventive prospective, in addition to its existing utility as a medicine for treating several diseases in the traditional medicine system of India, Ayurveda. Advance researches on hepatic detoxifying and anti oxidant enzymes by the extract of the herb, E.alba on Swiss albino mice are in progress.

ACKNOWLEDGEMENT

Thanks are due to Dr. T. Rahman, Head of the Department of Pathology, College of Veterinary Sciences, Assam Agricultural University, for his generous help in the histopathological study of the papillomas.

REFERENCES


Kanjilal, UN 1997 Flora of Assam.Omsons Publications, New Delhi, India.


Kumar, S 2002. The medicinal plants of North-East India Scientific Publishers, Jodhpur, India.


Siddiqui, AM. and Pawar, SS. 1984. Effects of garlic oil administration on hepatic microsomal mixed function oxidase system in adult male and female rats. 53rd SBC meeting, Delhi, India.


