Chemomodulatory effect of *Dolichos biflorus* Linn. on skin and forestomach papillomagenesis in Swiss albino mice

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Effect of consumption of three different doses (2%, 4% and 6%, w/w) of *Dolichos biflorus* Linn. seeds on hepatic drug metabolizing enzymes, antioxidant enzymes, reduced glutathione content, lactate dehydrogenase and lipid peroxidation in Swiss albino mice has been reported. Anti-carcinogenic effect has been studied by 7,12-dimethylbenzanthracene (DMBA)-induced skin and benzo(a)pyrene [B(a)P]-induced forestomach papillomagenesis models. *D. biflorus* consumption resulted in a significant increase in hepatic carcinogen metabolizing enzyme systems especially at 4% and 6% doses. Significant increase in reduced glutathione content (GSH) and specific activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) in liver of mice, at 4% and 6% doses has been reported. Lactate dehydrogenase (LDH) activity and peroxidative damage has been significantly decreased at 4% and 6% doses. In skin papillomagenesis model, 4% and 6% dose in diet significantly reduced the tumor incidence (up to 25%), tumor multiplicity (up to 59%) and tumor volume per mouse (up to 70%) as compared to DMBA treated group. Importantly, significant reduction in tumor incidence (up to 33%) and tumor multiplicity (up to 61%) was evident for forestomach papillomagenesis model.

**Keywords:** Benzo(a)pyrene, Chemomodulation, *Dolichos biflorus*, Papillomagenesis, Peroxidative damage

*Dolichos biflorus* Linn. (Fabaceae) is commonly known as horse gram in English and traditionally as ‘Kulthi’ in Hindi. This plant is native of Southeast Asia and is now widely distributed throughout the tropics, especially India, Malaysia, Mauritius, West Indies, and in the Queensland, Australia. On an average seeds contains protein (22-24%), carbohydrates (57.2%), fat (1.1%), vitamin A (40-199 IU/100 g), thiamine (0.40 mg/100 g), riboflavin (0.15 mg/100 g) and 3.2% minerals (Ca-105 mg, Fe-11.9 mg, Mn-15 mg and Zn-3.4 mg/100 g). It is one of the major ingredients of Ayurvedic medicine called *Cystone*, and prescribed to patients suffering from kidney ailments³. Roots of the plant are used as expectorant in China⁴. The methanolic extract of entire plant is shown to possess considerable hypolipidemic and antioxidant potential⁵.

Natural products are known to modulate cellular detoxification system which provides protection against genotoxic effects of various environmental toxicants⁶. In particular, natural compounds with antioxidative and anti-inflammatory activities have been demonstrated as potent chemopreventive agent against multi-stage carcinogenesis⁷,⁸. Skin cancer accounts for 30% of all newly diagnosed cancers in the world. Epidemiological studies have established it as one the fastest growing type of cancer in the world with more than one million new cases every year in USA alone⁹,¹⁰. Stomach cancer is second most prevalent malignancy in the world and the chemoprevention has evolved as an effective strategy to combat this dreadful disease¹¹.

7,12-dimethylbenzanthracene (DMBA) and benzo(a)pyrene [B(a)P] are biproducts of fossil fuel burning and potential environmental carcinogen. The cytoplasmic S-epoxide transferase converts DMBA into DMBA-tans-3,4-dio-1,2-epoxide, which is known to form DNA adducts by binding to N⁶ position of deoxyadenosine, causing A:T-T:A transversion¹². Skin papillomas induced by DMBA have a characteristic A-T at second nucleotide of codon 61 of the H-Ras gene¹³. Topical application of Croton oil, especially its main constituents like 12-O-tetradecanoylphorbol-13-acetate (TPA) cause many histological and biochemical changes in mouse skin. It induce skin edema, epidermal hyperplasia, inflammation, induction of epidermal lipoxygenase.

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and cyclooxygenase, oxidative stress causing increased H$_2$O$_2$ formation and enhanced lipid peroxidative damage$^{14,15}$. B(a)P also undergoes metabolic activation and subsequent epoxidation by microsomal enzymes to form anti-7,8-dihydroxy-9,10-epoxy, 7,8,9,10-tetrahydrobenzopyrene (anti-BPDE), which is ultimate carcinogen damaging DNA by virtue of its capacity to form DNA adducts, predominantly at N$^2$ position of guanine to form N$^2$-BPDE-deguanosine adduct$^{16}$.

In the present study cancer chemopreventive potential of $D$. biflorus seed diet on DMBA-induced skin and B(a)P-induced forestomach papillomagenesis in mice has been investigated. $D$. biflorus seed diet was also assessed for its capacity to modulate the levels/activities of mice hepatic phase I and phase II enzymes, antioxidant enzymes and content of reduced glutathione. The inhibitory effect on peroxidative damage and LDH activity was also examined.

**Materials and Methods**

**Chemicals**—Benzo(a)pyrene [B(a)P], 7,12-dimethylbenzanthracene (DMBA), potassium ferricyanide, thiobarbituric acid (TBA) and Triton X-100 were obtained from Sigma Chemical Co., USA. Rest of the chemicals were of highest purity and obtained from local firms in India.

**Animals**—Random-bred male Swiss albino mice (6-8 weeks old) were used in the present study. They were maintained on standard food pellets and water *ad libitum*. The experimental protocol was approved by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India and Institutional Animal Ethics Committee (IAEC), Jawaharlal Nehru University, New Delhi.

**Preparation of test diet**—Seeds of $D$. biflorus were crushed to powder and mixed with standard feed powder to prepare the pellets of desire doses of test diet viz. 2%, 4% and 6% (w/w). Air dried pellets were stored in neat, clean bags in the feed store-room in the animal house of Jawaharlal Nehru University and freshly prepared every week.

**Effect of Dolichos biflorus on xenobiotics metabolizing enzymes, antioxidants profile, lactate dehydrogenase and peroxidative damage in liver of mice**—Animals were randomly assorted on the basis of body weight into 4 groups (I-IV) comprising of 8 animals each. Animals in Group I served as untreated control, animals in Groups II, III and IV were kept on 2%, 4% and 6% doses of $D$. biflorus diet respectively, for 2 weeks and sacrificed after overnight fasting.

**Preparation of homogenate, cytosol and microsomal fractions**—Animals were sacrificed by cervical dislocation and the entire liver was then perfused immediately with ice-cold saline (0.9% NaCl) and rinsed in chilled 0.15 M Tris-KCl buffer (pH 7.4). Liver homogenate (10%) was prepared in ice-cold 0.15 M Tris-KCl buffer (pH 7.4). Further preparation of hepatic cytosolic and microsomal fraction was carried out as previously described$^{17}$. Protein content was determined according to the method of Lowry et al.$^{18}$ using BSA as standard.

**Assay methods**—All the biochemical assays were carried out according to the procedures described in Singh and Kale$^{17}$. Briefly, the Cyt P450 was measured by method of Omura and Sato$^{19}$. Specific activity of NADPH-cytochrome P450 reductase (Cyt P450R) was carried out according to the method of Omura and Takesue$^{20}$. NADH-cytochrome b5 reductase (Cyt b5R) activity was determined according to the method of Mihara and Sato$^{21}$ in microsomal fraction, and phase II cytosolic enzyme GST activity was determined according to the method of Habig et al.$^{22}$ and DTD activity was measured as described by Ernster et al.$^{23}$.

The GSH content was estimated by method of Moron et al.$^{24}$, GPX activity was measured by assay method described by Paglia and Valentine$^{25}$ and GR activity was determined by the method of Carlberg and Mannervik$^{26}$. SOD activity was assayed by the method of Marklund and Marklund$^{27}$ and catalase (CAT) activity was assayed by the method of Aebi$^{28,29}$. LDH was assayed according to the method of Bergmeyer and Bernt$^{30}$ and peroxidative damage in microsomes was estimated by the method of Varshney and Kale$^{31}$.

**Effect of Dolichos biflorus on DMBA-induced skin papillomagenesis**—DMBA-induced skin papillomagenesis was carried out according to method described in Yasukawa et al.$^{32}$. The mice were randomized by weight into five groups (I, II, III, IV and V) comprising 16 animals each group. Group I served as negative control, group II served as carcinogen (DMBA) treated positive control, groups III, IV and V were treated with 2%, 4% and 6% doses of $D$. biflorus test diet respectively, starting two
weeks before DMBA application and continued until the termination of experiment. After two weeks animals in all the groups were shaved on the dorsal surface approximately 6 cm². DMBA (50 μg/50 μl in acetone) was applied after 48 h on shaved dorsal skin of the animals except group I. One week later, each mouse in groups II, III, IV and V was topically treated with 0.1 ml of 1% Croton oil (dissolved in acetone) twice weekly for 16 weeks. Tumors of size >1mm in diameter were included in the cumulative total if they persisted for two weeks or more. Body weight gain profile, tumor incidence (% tumor bearing animals/group), tumor multiplicity (average number of tumors/mouse), tumor latency in days, and tumor volume (mm³) calculated by using the formula V= 4/3 πr³, where r is the radius of the tumor, were recorded for each animal.

Effect of Dolichos biflorus on B(a)P-induced forestomach papillomagenesis—This experiment was carried out as described by Azuine and Bhide. The animals were randomized by weight into five groups (I, II, III, IV and V) comprising of 18 animals each group. Animals in group I served as vehicle treated control, group II served as carcinogen [B(a)P] treated positive control, groups III, IV and V were treated with 2%, 4% and 6% doses of D. biflorus test diet respectively, starting two weeks before carcinogen application and continued until termination of experiment. Two weeks later animals in groups II, III, IV and V received 1 mg B(a)P/0.1 ml (dissolved in peanut oil) through oral gavage twice weekly for four weeks. Duration of this experiment was 24 weeks. Body weight was recorded on the weekly basis. At termination, animals were sacrificed by cervical dislocation and forestomach tissue was cut-open. Tumors were observed and counted under dissection microscope. Tumor incidences and tumor multiplicity were recorded for each animal.

Histopathology—At the termination of experiments, the skin and forestomach tumor samples from different treatment groups were fixed in 10% formalin. Following standard routine histochemistry techniques, the tissues were finally embedded in paraffin wax and sections 4-5 micron thick sections were cut and stained in hematoxylin and eosin for histological study.

Statistical analysis—Experimental values are given as mean ± SD. Statistical significance of difference between control and experimental groups was assessed by One-way ANOVA followed by Tukey’s Multiple Comparison test. A value of P<0.05 was considered statistically significant.

Results and Discussion

Effect of D. biflorus on phase I and phase II carcinogen metabolizing enzymes—D. biflorus diet, particularly its 4% and 6% doses, significantly enhanced the various components of both phase I and phase II system in mice liver. Relative to control, significant increase in Cyt P450 content and activity of Cyt P450R and Cyt b5R was evident at 4% and 6% doses. The activity of GST showed a dose dependent increase by 19%, 28% and 33% over control group with consumption of 2%, 4% and 6% doses of D. biflorus, respectively. Compared to control, the DTD activity was also increased significantly by 25% and 40% at 4% and 6% doses, respectively (Table 1).

Modulatory effect of Dolichos biflorus on antioxidant system and toxicity parameters—GSH content was significantly elevated by 35% and 56% at 4% and 6% doses of D. biflorus, respectively. The activity of GPX was increased by 22% and 24% by 4% and 6% doses, respectively. GR was significantly enhanced by 21% and 36% in animals treated with 4% and 6% doses of given test diet, respectively. Relative to control, the activity of SOD showed an increase with 4% and 6% doses of D. biflorus diet by 17% and 32%, respectively. All the investigated doses of D. biflorus diet could also enhance the activity of catalase by 21%, 34% and 26%, respectively. Assaying toxicity parameters, LDH activity was significantly inhibited by 24% and 23%, at 4% and 6% dose of D. biflorus, respectively. In case of formation of MDA, measured as index of peroxidative damages, was found to be effectively decreased to 12% and 14% (Table 1).

Modulatory effect of Dolichos biflorus on DMBA-induced skin papillomagenesis—At the termination of experiment after 16 weeks all the animals treated with different doses of D. biflorus diet were found to be healthy, suggesting no toxic effect of its long-term consumption. Compared to DMBA treated positive control, the body weight of animals receiving 4% and 6% doses of test diet was significantly increased (Table 2). Moreover, no evidence of development of spontaneous tumours, including skin lesion were encountered in animals in Group I (Negative control). DMBA caused 100% incidence of skin tumour in animals of group II (positive control). When tested, the diet of D. biflorus was found to have
chemopreventive effect particularly at higher doses. It was evident from the animals treated with 4% and 6% doses of diet respectively, which accounted for 18.75% and 25% reduction in tumour incidence. However, lower dose (2% test diet) failed to provide chemopreventive effect. Compared DMBA treated group tumour multiplicity was significantly reduced by 2%, 4% and 6% diet of *D. biflorus* to 21%, 45% and 59%, respectively. Moreover, a dose dependent delay in appearance of first tumour in *D. biflorus* diet treated groups was evident when compared to positive control, thus enhancing tumour latency (in days) by 9%, 22% and 28% with 2%, 4% and 6% doses, respectively. Significant reduction was found in tumour volume per mouse by 23%, 50% and 70% respectively (Table 2).

Table 1—Effect of *Dolichos biflorus* test diet on body weight, phase I and phase II enzymes, antioxidant parameters, LDH activity and peroxidative damage in the liver of mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th><em>D. biflorus</em> (2%)</th>
<th><em>D. biflorus</em> (4%)</th>
<th><em>D. biflorus</em> (6%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>24.33 ± 1.51</td>
<td>25.33 ± 1.63</td>
<td>24.67 ± 2.10</td>
<td>25.67 ± 1.51</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>28.67 ± 1.63</td>
<td>29.00 ± 1.67</td>
<td>29.67 ± 1.97</td>
<td>31.00 ± 2.14</td>
</tr>
<tr>
<td>Cyt P450 (nmol/mg protein)</td>
<td>0.392 ± 0.021</td>
<td>0.421 ± 0.032</td>
<td>0.435 ± 0.018</td>
<td>0.441 ± 0.028</td>
</tr>
<tr>
<td>Cyt P450 R (µmole of NADPH oxidized/min/mg protein)</td>
<td>0.180 ± 0.018</td>
<td>0.191 ± 0.020</td>
<td>0.211 ± 0.025</td>
<td>0.215 ± 0.022</td>
</tr>
<tr>
<td>Cyt b5 R (µmole of NADH oxidized/min/mg protein)</td>
<td>3.18 ± 0.195</td>
<td>3.39 ± 0.209</td>
<td>3.51 ± 0.231</td>
<td>3.60 ± 0.244</td>
</tr>
<tr>
<td>GST (µmole CDNB-GSH conjugated/min/mg protein)</td>
<td>1.99 ± 0.184</td>
<td>2.37 ± 0.197</td>
<td>2.55 ± 0.258</td>
<td>2.64 ± 0.367</td>
</tr>
<tr>
<td>DTD (µmole DCPIP reduced/min/mg protein)</td>
<td>0.020 ± 0.002</td>
<td>0.023 ± 0.001</td>
<td>0.025 ± 0.005</td>
<td>0.028 ± 0.003</td>
</tr>
<tr>
<td>GPX (nmole of NADPH oxidized/min/mg protein)</td>
<td>28.35 ± 3.67</td>
<td>32.15 ± 3.03</td>
<td>34.60 ± 2.80</td>
<td>35.28 ± 3.45</td>
</tr>
<tr>
<td>GR (nmole of NADPH oxidized/min/mg protein)</td>
<td>21.65 ± 2.61</td>
<td>23.55 ± 2.00</td>
<td>26.35 ± 3.52</td>
<td>29.54 ± 2.37</td>
</tr>
<tr>
<td>SOD (µmole/mg protein)</td>
<td>5.19 ± 0.38</td>
<td>5.68 ± 0.42</td>
<td>6.09 ± 0.64</td>
<td>6.83 ± 0.51</td>
</tr>
<tr>
<td>CAT (µmole H₂O₂ consumed/min/mg protein)</td>
<td>69.57 ± 5.96</td>
<td>84.37 ± 10.24</td>
<td>93.31 ± 12.35</td>
<td>87.62 ± 9.95</td>
</tr>
<tr>
<td>GSH (nmole GSH/gm tissue)</td>
<td>2.34 ± 0.26</td>
<td>2.85 ± 0.38</td>
<td>3.15 ± 0.49</td>
<td>3.24 ± 0.51</td>
</tr>
<tr>
<td>LDH (µmole NADH oxidized/min/mg protein)</td>
<td>2.25 ± 0.32</td>
<td>1.88 ± 0.25</td>
<td>1.74 ± 0.27</td>
<td>1.72 ± 0.38</td>
</tr>
<tr>
<td>Peroxidative damage (nmole MDA formed/mg protein)</td>
<td>0.87 ± 0.071</td>
<td>0.81 ± 0.064</td>
<td>0.77 ± 0.041</td>
<td>0.75 ± 0.049</td>
</tr>
</tbody>
</table>

*P* values: *a*<0.05, *b*<0.01 and *c*<0.001 represent significant change over control.

Modulatory effect of *Dolichos biflorus* on *B(a)P*-induced forestomach papillomagenesis—All the animals were found to be healthy at termination of experiment and a significant gain of 1.07-fold was observed in body weight of animals treated with 6% dose (Group V) when compared to positive control (Table 3). No apparent sign of toxicity was found on internal viscera. Moreover, no evidence of development of spontaneous tumour lesions was encountered in negative control (Group I). All the animals in *B(a)P*-treated positive control developed forestomach tumours. 2%, 4% and 6% doses decreased the incidence of tumours by 16%, 28% and 33%, respectively. Compared to positive control (Group II) tumor multiplicity was reduced...
by 40%, 54% and 61% with 2%, 4% and 6% doses of *D. biflorus*, respectively.

**Histopathological studies**—Histological section of the skin from the negative control group of animals exhibited a normal histological appearance showing flattened epidermis, and dermis showing dense population of hair follicles without any signs of pathology (Fig. 1A). On the other hand, a section from the animals treated with DMBA+Croton oil (positive control) showed the development of benign squamous papillomas arising from the epidermis showing dysplasia as well as benign keratin pearls (Fig. 1B). The section of skin from mice treated with 2% dose of *D. biflorus* showed remarkably thick epidermis as well as hyperplasia and dermis was unremarkable (Fig. 1C). Significant reduction in hyperplasia and epidermal thickness was visible with 4% dose of *D. biflorus* (Fig. 1D), thus demonstrating dose dependent chemopreventive effect of *D. biflorus* on skin papillomagenesis.

![Histopathological studies](image)

Table 2—Effect of *Dolichos biflorus* test diet on DMBA-induced Skin Papillomagenesis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acetone</th>
<th>DMBA+ croton oil</th>
<th>DMBA+ croton oil + 2% <em>D. biflorus</em></th>
<th>DMBA+ croton oil + 4% <em>D. biflorus</em></th>
<th>DMBA+ croton oil + 6% <em>D. biflorus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>24.00 ± 2.93</td>
<td>26.33 ± 2.59</td>
<td>23.50 ± 2.87</td>
<td>25.67 ± 2.46</td>
<td>24.85 ± 1.84</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>41.22 ± 2.92</td>
<td>39.15 ± 2.87</td>
<td>41.37 ± 3.22</td>
<td>42.35 ± 2.65^a</td>
<td>43.11 ± 3.64^b</td>
</tr>
<tr>
<td>Tumor incidence</td>
<td>Nil</td>
<td>100%</td>
<td>100%</td>
<td>81.25%</td>
<td>75%</td>
</tr>
<tr>
<td>Tumor multiplicity</td>
<td>Nil</td>
<td>8.25 ± 2.84</td>
<td>6.50 ± 2.50</td>
<td>4.50 ± 2.73^c</td>
<td>3.38 ± 1.75^c</td>
</tr>
<tr>
<td>Tumor latency (in days)</td>
<td>Nil</td>
<td>46.80 ± 10.89</td>
<td>51.19 ± 11.43</td>
<td>57.15 ± 12.39</td>
<td>59.88 ± 12.79^a</td>
</tr>
<tr>
<td>Tumor volume/mouse (mm^3)</td>
<td>Nil</td>
<td>457 ± 38</td>
<td>352 ± 24^c</td>
<td>229 ± 15^c</td>
<td>136 ± 12^c</td>
</tr>
</tbody>
</table>

^P values: ^a^<0.05 and ^c^<0.001 represents significant change over DMBA+ croton oil treated group.

Table 3—Effect of *Dolichos biflorus* test diet on B(a)P-induced Forestomach Papillomagenesis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vehicle</th>
<th>B(a)P</th>
<th>B(a)P + 2% <em>D. biflorus</em></th>
<th>B(a)P + 4% <em>D. biflorus</em></th>
<th>B(a)P + 6% <em>D. biflorus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>25.18 ± 2.42</td>
<td>24.65 ± 2.61</td>
<td>25.60 ± 2.23</td>
<td>26.48 ± 2.22</td>
<td>24.37 ± 2.90</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>38.89 ± 3.31</td>
<td>39.22 ± 3.30</td>
<td>40.44 ± 2.79</td>
<td>41.00 ± 2.59</td>
<td>42.00 ± 2.47^a</td>
</tr>
<tr>
<td>Tumor incidence</td>
<td>Nil</td>
<td>100%</td>
<td>83.33%</td>
<td>72.22%</td>
<td>66.67%</td>
</tr>
<tr>
<td>Tumor multiplicity</td>
<td>Nil</td>
<td>4.56 ± 1.92</td>
<td>2.72 ± 1.96^a</td>
<td>2.11 ± 1.64^c</td>
<td>1.78 ± 1.73^c</td>
</tr>
</tbody>
</table>

^P values: ^a^<0.05 and ^c^<0.001 represents significant change over B(a)P treated group.

Histological sections from the forestomach of negative control group exhibited normal histology with keratinized epithelium on top of submucous and muscle layer (Fig. 2A). Section from the animals treated with B(a)P exhibited development of benign squamous papilloma with simple branching arising from the normal gut lining (Fig. 2B). With respect to B(a)P treated group the sections from animals treated with different doses of *D. biflorus* diet exhibited a decrease in size and branching of papilloma, maximum with 6% dose of *D. biflorus*. These findings for 2% and 4% doses are shown in Fig. 2C and Fig. 2D respectively.

Several experimental and epidemiological studies have shown that chemoprevention has the potential of providing an important means for cancer prevention, importantly for the individuals at high risk of developing cancer. Thus screening of natural products, especially diet based compounds has great potential to emerge as potent cancer preventive...
compound due to their efficacy and non-toxicity at higher doses\textsuperscript{36}. To exert mutagenic effect, polyaromatic hydrocarbons like DMBA and B(a)P undergo metabolic activation by phase I enzymes into diol-epoxides which interact with DNA and in turn may transform the cell into tumorigenic one. The relatively more polar and electrophilic metabolites like diol-epoxides are further metabolized by phase II system rendering them to be more hydrophilic products which in turn are excreted from the body. \textit{D. biflorus} diet was able to enhance the profile of phase I system like Cyt P450 content and activity of Cyt P450R and Cyt b5R. At the same time it also enhanced the activities of phase II enzymes GST and DTD, which are expected to detoxify the mutagenic metabolites ultimately leading in part to inhibition of tumorigenesis\textsuperscript{37,38}. As \textit{D. biflorus} has enhanced the activities of both phase I and II enzyme systems, it could be considered as bifunctional inducer\textsuperscript{39}.

It is well established that free radicals are involved in cause and complication of cancer especially at tumor promotional stage. Therefore, any modulator which has ability to scavenge free radicals or interfere with the free radical dependent process is expected to inhibit the tumorigenesis. \textit{D. biflorus} diet could significantly increase the activities of antioxidant enzymes such as SOD, CAT, GPX and GR, which are expected to scavenge the free radicals generated by application of tumor promoters like Croton oil. Enhanced levels of GSH content is expected to scavenge free radicals directly or participate in reactions of peroxidase contributing to protection against tumorigenesis. A significant inhibition of specific activity of LDH and peroxidative damages supports the possibilities of involvement of antioxidant system in protecting animals against tumorigenesis\textsuperscript{37,38}.

Fig. 1—Histological study of skin papillomas: (A)-acetone group showing normal epidermal and dermal layers; (B)-DMBA+Croton oil (positive control) showing benign squamous papillomas; (C)-\textit{D. biflorus} (2\%) treated mice tumor section showing epidermal and dermal hyperplasia and; (D)-mice treated with 4\% dose of \textit{D. biflorus} showing reduction in pathology of skin (→), H & E 100 ×.
In the present study, dietary consumption of *D. biflorus* exerted a chemopreventive effect against DMBA-induced skin papillomagenesis in Swiss albino mice. It is evident from decrease in tumor incidences, tumor multiplicity and tumor volume (Table 2). For wide acceptability, it is desirable that a plant or plant product is effective against more than one type of cancer. For this, we used forestomach papillomagenesis as a model. *D. biflorus* diet was also effective in providing protection against B(a)P-induced forestomach papillomagenesis in mice, which resulted in inhibition in both tumor incidence and tumor multiplicity (Table 3). The histopathological findings are also supportive of our study. Earlier studies by Muthu *et al.*⁴⁰ are suggestive of the presence of antioxidants in the *D. biflorus*. These antioxidant phytochemicals might have scavenged the free radicals formed during the metabolism of carcinogens and contributed to chemomodulatory effect. Further, the antioxidant phytochemicals might have also influenced the metabolism of DMBA and B(a)P through modulatory effect on the phase I and phase II enzymes systems leading to detoxification of their carcinogenic metabolites. The findings of this study may have significance to reduce the incidences of cancer especially in the high risk groups in human population.

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**References**


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Fig. 2—Histological sections of forestomach papillomas obtained from different treatment groups: (A)-normal epithelium in vehicle treated group; (B)-B(a)P only (positive control); (C)- 2% dose of *D. biflorus* and; (D)-4% dose of *D. biflorus* showing reduction in papillomas of forestomach tissue (→), H & E 100×.
490


