Antioxidant and anticarcinogenic activity of Lycovin - an indigenous herbal preparation

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Aqueous extract of Lycovin has been found to be a potent inhibitor of lipid peroxide formation, (IC_{50}=500 \mu g/ml) and scavenger of hydroxyl radical (IC_{50}=44 \mu g/ml) and superoxide radical (IC_{50}=30 \mu g/ml) in vitro. Lycovin syrup 1.5 ml and 7.5 ml/kg body wt administered orally, reduced the development of sarcoma induced by 20 MC by 35% and 70% respectively. Lycovin syrup was also found to inhibit the hepatocarcinogenesis induced by NDEA. The tumour incidence was 100% in the control group, while none of the drug treated animals developed tumour. Liver weight, γ-glutamyl transpeptidase (GGT), GSH-S-transferase (GST), reduced glutathione, (GSH) and aniline-4-hydroxylase in liver were elevated in NDEA alone treated animals. The serum parameters indicative of liver injury such as bilirubin, lipid peroxides, alkaline phosphatase and glutamate pyruvate transaminase were also elevated by NDEA administration. These elevated parameters were significantly reduced in animals treated with Lycovin syrup along with NDEA in a dose dependent manner. Even though the exact mechanism of action is not known at present, the observed antioxidant activity may be due to the inhibition of P.450 enzyme activity and subsequent inhibition of the production of the ultimate carcinogen as well as scavenging of oxygen free radicals during promotion of the transformed cell.

Role of oxygen radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, ageing etc. and the drugs that can scavenge oxygen radicals have great potential in ameliorating these disease processes. Although the body has several mechanism to protect the action of oxygen radicals at times these protective mechanisms are found to be not sufficient when compared to the insult produced to the body. Supplementation of non-toxic antioxidants may have a chemoprotective role in the body in these conditions.

Lycovin, a herbal preparation has been reported to possess a beneficial effect in case of hepatotoxicity induced by CCl_{4} and thioacetamide (unpublished data). Lycovin syrup contains the following indigenous plant extracts reported in the ancient system of Ayurveda medicine to be of value in the management of liver disorders: Pierorrhiza kurroa (5 g), Phyllanthus amarus, (400 mg), Boerhaavia diffusa (100 mg), Berberis aristata (50 mg), Cichorium intybus (150 mg), Eclipta alba (200 mg), Terminalia chebula (50 mg) and Ricinus communis (100 mg) in 5 ml. In the present report we provide evidence that Lycovin, an indigenous herbal preparation have potent in vitro antioxidant activity and has anticarcinogenic activity in animal models.

Materials and Methods

Animals—Swiss albino mice were purchased from the National Institute of Nutrition, Hyderabad, India. Male Wistar rats were purchased from Veterinary College, Mannuthy. They were housed in ventilated cages and fed with a pellet diet (Lipton, India Ltd.) and water ad libitum.

Materials—Lycovin syrup and capsules were supplied by Dr.S.S.Gandhi, Medical Advisor, Lyka Research Labs, Bombay; N-nitrosodiethyamine (NDEA), L-glutamyl-p-nitroanilide, glycylglycine were purchased from Sigma chemicals, St.Louis M.O, USA. 20-Methylcholanthrene (20 MC) was purchased from ICN-Pharmaceuticals, New York, NY. NADPH, 5,5'Dithio bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), dimethyl sulfoxide (DMSO) and aniline were purchased from Sisco Research Laboratories, Bombay. 1-Chloro-2,4-dinitrobenzene (CDNB) from Loba Chemie Pvt Ltd, Bombay. Thiobarbituric acid was obtained from E.Merck (India). All other chemicals used were of analytical reagent grade.

Extraction of Lycovin powder—Each time Lycovin powder (50 gm), was extracted twice with 500 ml of hot water by stirring overnight and centrifuged at room temperature. The supernatant was collected and evaporated to dryness. Yield was
This dosage has been reported to produce sarcoma in DMSO/mice. Hair was removed from methylcholanthrene-induced sarcoma-Male sacrificed by diethylether anaesthesia, blood and liver sacrificing. At the 21st week, kept without any treatment for 5 days prior to the first dose syrup was given starting 5 days before the first dose. Lycovin was non-toxic to animals. Lycovin liver was kept frozen until analysed.

III and IV were given two different concentrations of Lycovin syrup (1.5 ml and 7.5 ml/kg body wt) along with the NDEA. This dosage was arbitrarily calculated from the proposed human dosage. At this dosage Lycovin was non-toxic to animals. Lycovin syrup was given starting 5 days prior to the first dose of NDEA and continued for 20 weeks. All rats were kept without any treatment for 1 week before sacrificing. At the 21st week, all animals were sacrificed by diethylether anaesthesia, blood and liver were collected immediately. Serum was separated and liver was kept frozen until analysed.

Effect of Lycovin syrup on 20-methylcholanthrene induced sarcoma—Male Swiss albino mice (20-25 g), were used for this study. Hair was removed from the dorsal side of all animals and single dose of 20-methylcholanthrene (20 MC) (200 μg/0.1ml DMSO/mice) was injected (sc) on the dorsal side. This dosage has been reported to produce sarcoma in these animals. Animals were divided into three groups (20/group). Group I, served as control group kept without any drug treatment after 20 MC injection. Different concentrations of Lycovin; 1.5 ml and 7.5 ml/kg body wt was given orally to group II and III respectively thrice a week for 8 weeks. This time schedule was similar to that for Emblica officinalis reported earlier. The animals were observed for the onset of sarcoma as well as for their survival up to 180 days. The following biochemical parameters were carried out.

Lipid peroxide level in serum was estimated by the thioarbituric acid method and expressed in terms of malonaldehyde. Protein was analysed by the method of Lowry et al. Glutamate pyruvate transaminase (GPT) activity assay was done by Bergmeyer and Bernt method. Alkaline phosphatase (ALP) assay was done according to King and Armstrong method. Cytosolic glutathione-S-transferase (GST) activity and reduced glutathione levels (GSH) were measured by the procedures of Habig et al. and Moron et al., respectively. Serum bilirubin was estimated by standard method. Serum γ-Glutamyl transpeptidase (GGT) was estimated by Szasz's method and tissue GGT by using P-nitroanilide as substrate. Aniline hydroxylase activity was assayed by Mazel's method. The statistical difference was analysed by Student's t test.

Results

Effect of Lycovin extract on generation of oxygen free radicals in vitro—Extracts of Lycovin was found to scavenge the superoxide, hydroxyl radicals and lipid peroxidation in vitro. (Fig.1). The concentration of Lycovin needed for 50% scavenging of superoxide generated by photoreduction of riboflavin was found to be 30 μg/ml. Degradation of

![Fig. 1—Effect of Lycovin on inhibition of lipid peroxidation (●●); superoxide generation (Δ-Δ), and hydroxyl radical (○○)](image-url)
deoxyribose mediated by hydroxyl radicals generated by the Fe³⁺/ascorbate/EDTA/H₂O₂ system was also found to be inhibited by the addition of Lycovin. The concentration of the Lycovin needed for 50% inhibition was 44 µg/ml. The concentration of the Lycovin needed for 50% inhibition of lipid peroxidation was found to be 500 µg/ml. Observed antioxidant property was almost the average of antioxidant activity of the individual extracts present in the Lycovin (data not given).

Effect of Lycovin syrup on development of hepatocarcinoma—Lycovin syrup was found to inhibit hepatocarcinogenesis induced by NDEA significantly (Table I). Control animals developed frank hepatomas after about 18-20 weeks of carcinogen feeding. The incidence of liver tumour in the NDEA alone treated group was 100%. Administration of Lycovin reduced the tumour incidence completely. The average liver weight of the NDEA alone treated animals increased from 2.78±0.04 to 7.59±0.61 g/100g body wt (Table I) when the animals were treated with Lycovin, the liver weight was almost maintained to the normal level.

γ-Glutamyl transeptidase (GGT) enzyme activity which is a biomarker for hepatocarcinogenesis was found to be significantly elevated from 32.8±2.3 to 130.6±13.3 U/L in serum and from 0.08±0.02 to 2.93±0.08 nmol/min/mg protein in liver by the administration of NDEA. In Lycovin treated groups GGT in serum and liver were found to be significantly reduced (Table I). The effect was dose dependent.

Glutathione-S-transferase activity, and reduced glutathione levels in liver were significantly increased by 135% and 116% respectively by the administration of NDEA (Table I). Administration of Lycovin, 1.5 ml/kg body wt reduced these elevated value significantly (P<0.001). These parameters were found to be further decreased at higher concentration of Lycovin syrup P<0.001 (Table 2). Aniline hydroxylase activity, a P-450 dependent enzyme, in normal liver was 0.212±0.08 and this value was significantly increased to 0.465±0.06 µmol/min/mg protein by the administration of NDEA. Administration of Lycovin syrup (1.5 ml/kg body wt), reduced the level to 0.389±0.03 µmol/min/mg protein. This was further decreased to 0.291±0.05 at higher concentration of Lycovin (7.5 ml/kg body wt) (Table 2).

Lipid peroxide level, GPT and ALP in the normal rat serum were found to be 1.89±0.09 nmol/ml, 215.4±19.7 U/ml, 17.2±0.1 KA/100 ml respectively. These were elevated to 4.04±0.49 nmol/ml, 723.2±144 U/ml, and 48.8±10.6 KA/100 ml respectively after the administration of NDEA. Administration of Lycovin, significantly lowered the lipid peroxide level GPT and ALP in a dose

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Dose/kg body wt)</th>
<th>No. of tumour bearing animals</th>
<th>Tumour incidence %</th>
<th>Liver wt %</th>
<th>GST (nmol/min/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>Aniline hydroxylase (µmol/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>0/8</td>
<td>0</td>
<td>2.78±0.04</td>
<td>32.8±2.3</td>
<td>0.08±0.02</td>
<td>0.212±0.08</td>
</tr>
<tr>
<td>II</td>
<td>NDEA alone (Control)</td>
<td>8/8</td>
<td>100</td>
<td>7.59±0.61</td>
<td>130.6±13.3</td>
<td>0.293±0.08</td>
<td>0.465±0.06</td>
</tr>
<tr>
<td>III</td>
<td>Lycovin syrup; 1.5 ml+NDEA</td>
<td>0/8</td>
<td>0</td>
<td>2.90±0.27*</td>
<td>49.2±6.6*</td>
<td>1.85±0.09*</td>
<td>0.389±0.03</td>
</tr>
<tr>
<td>IV</td>
<td>Lycovin syrup; 7.5 ml+NDEA</td>
<td>0/8</td>
<td>0</td>
<td>2.89±0.25*</td>
<td>46.5±3.7*</td>
<td>0.60±0.12*</td>
<td>0.291±0.05</td>
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*P<0.001

Table 2—Effect of Lycovin syrup on liver glutathione-S-transferase (GST) activity, reduced glutathiones (GSH) levels and aniline hydroxylase in rats treated with NDEA

[Values are mean±SD from 8 animals in each group]
Several diseases. Countries such as India and China have a large number of traditional medicines which have not yet been explored scientifically. These medicines are prescribed not only to reduce suffering but also to prevent diseases produced by pathophysiological changes. Even though cancer is one of the most difficult diseases to be treated, its prevention could be achieved by (a) avoidance of cancer-inducing substances; (b) chemopreventive agents that can inhibit the metabolism of carcinogen or cause its detoxification; (c) immunostimulators which can destroy cancer cells by augmenting the immune responses; (d) inhibition of signal transduction pathway which can either inhibit the conversion of normal cells to cancer cells, reduce its growth capability and destroy the cells by increasing the recognition by the immunocompetent cells.

Several types of compounds numbering more than 2000 chemicals among which many of them are from plant origin, have been reported to inhibit chemically induced carcinogenesis. The mechanism of several chemopreventive agents is still not clear. During the treatments with an extract like the ones utilised in this case, we could expect several chemical compounds capable of reducing the neoplastic formation at the different points discussed above.

The present data indicating that Lycovin extract has strong antioxidant activity in vitro provides a scientific explanation for the observed medicinal properties of the preparation. The present study indicates that a preparation of indigenous origin has dependent manner (Table 3). This was also reflected in liver lipid peroxides, GPT and ALP values (data not included). Serum bilirubin (Total as well as direct) which is a biomarker for liver damage, was significantly increased (P<0.001) by the administration of NDEA. This was almost equal to normal values (Table 3) in the Lycovin treated group (7.5 ml/kg body wt).

Effect of Lycovin syrup on development of 20-methylcholanthrene induced sarcoma—The effect of Lycovin on development of sarcoma induced with 20-methylcholanthrene is shown in Table 4. The animals in the control group started developing sarcomas 52 days after carcinogen administration and all the animals developed sarcoma within 120 days whereas in the Lycovin; 1.5 ml and 7.5 ml/kg body wt inhibited the sarcoma development by 35% and 70% respectively (Table 4). Lycovin syrup was also found to increase the life span of the animals treated with 20-methylcholanthrene (Table 4). Control animals started to die of their tumour burden after 71 days of carcinogen treatment, and all the animals were dead by 149 days. Whereas in the Lycovin treated group i.e. 1.5 ml and 7.5 ml/kg body wt; the survival of animals increased by 40% and 80% respectively up to 180 days.

Discussion

Herbal preparations are being used in alleviating several diseases. Countries such as India and China have large number of traditional medicines which have not yet been explored scientifically. These medicines are prescribed not only to reduce suffering but also to prevent diseases produced by pathophysiological changes. Even though cancer is one of the most difficult diseases to be treated, its prevention could be achieved by (a) avoidance of cancer-inducing substances; (b) chemopreventive agents that can inhibit the metabolism of carcinogen or cause its detoxification; (c) immunostimulators which can destroy cancer cells by augmenting the immune responses; (d) inhibition of signal transduction pathway which can either inhibit the conversion of normal cells to cancer cells, reduce its growth capability and destroy the cells by increasing the recognition by the immunocompetent cells.

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<th>Table 3 — Effect of Lycovin syrup on lipid peroxide; glutamate pyruvate transaminase (GPT), alkaline phosphatase (ALP) and bilirubin in serum of rats treated with NDEA</th>
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<tbody>
<tr>
<td>Treatment</td>
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<tr>
<td>I Normal</td>
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<td>II Control</td>
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<tr>
<td>III Lycovin; 1.5 ml+NDEA</td>
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*P<0.005 **P<0.001

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<th>Table 4 — Effect of Lycovin syrup on 20-methylcholanthrene (20 MC) induced sarcoma development and survival of animals</th>
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<tr>
<td>Treatment</td>
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been shown to inhibit chemically induced carcinogenesis very effectively both in methyl cholangthrene induced sarcoma as well as NDEA induced hepatocarcinogenesis.

Acknowledgement

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References