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ANTICANCER ACTIVITY OF *SESBANIA GRANDIFLORA* EXTRACT AGAINST DIETHYL NITROSAMINE INDUCED HEPATIC CARCINOMA

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ABSTRACT

The present study was designed to evaluate the anticancer activity of Sesbania grandifloria extract against diethyl nitrosamine induced hepatic carcinoma. The ethanolic extract of *Sesbania grandiflora* was screened for its anticancer activity in diethyl nitrosamine (DEN) induced liver cancer in experimental rat. The plant extract was administered orally once a week, up to 30 days after DEN administration. The animals were sacrificed; blood sample and liver tissue were collected and used for enzyme assay such as, aspartate amino transferase (AST), alanine aminotransferase (ALT), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST). The liver marker enzymes AST and ALT produced significant results in the protective action. The antioxidant enzyme assay results concerning the improved activity of GPx, GST and CAT. These results concluded that enhanced levels of antioxidant enzyme and reduced amount of serum amino transaminase, which are suggested to be the major mechanisms of *Sesbania grandiflora* root extract in protecting the rat from hepatocarcinoma induced by DEN. These biochemical observations were supplemented by histopathological examination of liver sections. The ethanolic extract of *Sesbania grandiflora* possesses significant anticancer properties

INTRODUCTION:

Cancer is one of the leading causes of death in the world. Its high incidence and mortality and lack of effective treatment have spurred extensive research on chemoprevention. In fact, this is the second leading cause of death after cardiovascular diseases in India. The cancers such as lung, breast, colon, and stomach are the most common incidences worldwide.[1] Most commonly cancers associated with diet include esophageal, stomach, colon, liver and prostate. Furthermore, this is one of the most dreaded diseases of the 20th century and spreading with continuance and increasing incidence in 21st century. In the United States, as the leading cause of death, it accounts for 25% of all the deaths in humans.[2]

Liver cancer affects nearly 22,000 people in the United States and more than 18,000 deaths. Until recently, the U.S. Food and Drug Administration (FDA) had not approved any medications specifically for liver cancer to treat. The drug sorafenib (Nexavar) was approved by the FDA for people with hepatocellular carcinoma (HCC) (Lowell Anthony, MD). HCC is the fifth most common cancer and the third leading cause of cancer mortality in the world.[3] Approximately 5.6 lakh new cases are diagnosed each year and around 5.5 lakh deaths due to liver cancer occur mostly in developing countries.[4] Although 80% of new cases are detected primarily in developing countries, the prevalence of liver cancer is also rising in Japan, Western Europe, and the United states. It has been

estimated that there will be 21,370 new cases and 18,410 deaths in the US in 2008 due to liver cancer.[5] Liver cancer consists of several histological different primary hepatic malignancies, such as cholangiocarcinoma, hepatoblastoma and hemangiosarcoma, but HCC is by the most common type, accounting for 70–85% of cases.[6,7] Medicinal plants are frequently used by traditional healers to treat a variety of ailments and symptoms including diabetes and cancer. According to world health organization, over 80% of the world's populations rely upon such traditional plant-based systems of medicine to provide them with primary healthcare. The present study was carried out to determine the anticancer potential of *Sesbania grandifloria* extracts using diethyl nitrosamine (DEN)-induced mice model.

MATERIALS AND METHODS

Plant materials

Sesbania grandiflora were collected from in and around Anantapuramu district. The leaf were washed thoroughly with running tap water. Then the same was shade dried for 2–3 weeks and was grounded to moderately coarse powder.

Extraction procedure

The dried and powdered plant material was extracted using 75% V/V ethanol in soxhlet apparatus for 6 h. The extract was concentrated using a rotary evaporator at 40–50°C under reduced pressure.

Animals

Adult male 3 months old, weight vary from 180 to 200 g Wistar albino rat were housed in polypropylene cages. They were kept in an animal room with 12:12 h day-night cycle with temperature of 28°C ± 2°C and humidity of 45–60%. They were fed with a commercial pelleted mice chow and water ad libitum throughout the study. The study was conducted after obtaining institutional animal ethical committee clearance.

Chemicals

Diethyl nitrosamine was obtained from Sigma Aldrich Ltd. A dose of 20 mg/kg body weight in 0.9% w/v NaCl and was administered intraperitonially. At the end of the treatment (30 days), the experimental animals were deprived of food overnight. Blood was collected without anticoagulant was used for serum separation; finally animals were sacrificed by cervical dislocation. Liver were carefully dissected out and washed with ice-cold saline and preserved in 10% w/v formalin solution for the enzymatic analysis and histological studies. 1.0 g of the liver tissue was homogenized in 0.2 M phosphate buffer, pH 7.0 at 4°C

Protein estimation

The homogenates were centrifuged at 6500 × g for 10 min at 4°C. The supernatant was used for enzymatic assay. Biochemical assessments were performed with the supernatant of liver homogenate and serum. Total protein concentration was determined by the method of Lowry et al.[8] The protein value was expressed as mg/g of tissue sample.

Marker enzyme assays

Marker enzymes were assayed by standard methods. Catalase (CAT) was assayed by the method described by Luck.[9] Glutathione peroxidase (GPx) activity was assayed by the method of Rotruck et al.[10] The enzyme activity was expressed as nano moles/min/ml. aspartate amino transferase (AST) and alanine aminotransferase (ALT) activities were measured by the methods of Bergmeyer and Bernt.[11] A standard curve was obtained using different amounts of pyruvate and serum activity was expressed as units/ml. Glutathione-S-transferase (GST) was assayed by the method of Habig et al.[12] Activity of GST was expressed as moles of 1-chloro 2,4 dinitrobenzene-glutathione (GSH) conjugate formed min/mg of protein.

Statistical analysis: All statistical analysis was conducted using one-way ANOVA with Dunnett's posttest using Graph Pad InStat

version 3.00 for Windows, Graph Pad Software, San Diego, CA, USA.

RESULTS

A highly significant ($P < 0.01$) elevation in serum glutamate pyruvate transaminase (SGPT) activity was observed in DEN and DEN + EESG groups when compared with control rat, Whereas the DEN + Doxorubicin treated animals showed the low significant alteration with DEN and DEN + EESG groups (Table 11). A highly significant ($P < 0.01$) elevation in serum glutamate oxaloacetate transaminase (SGOT) activity was observed in DEN, DEN + EESG and DEN + Doxorubicin rat. The saline treated did not show the any significant alteration (Table 1).

Diethyl nitrosamine treated group showed low significant elevation ($P < 0.05$) in liver GST activity with respect to control, Whereas the control, DEN + EESG, DEN + Doxorubicin treated animals did not shown any alteration (Table 2). A highly significant ($P < 0.01$) elevation in GPx activity was observed in DEN treated rat, whereas DEN + EESG showed low significant alteration the same time saline and DEN + Doxorubicin treated animals did not show any significant alteration (Table 12). DEN, DEN + EESG showed low significant depletion ($P < 0.05$) in liver CAT activity with respect to control, whereas saline and DEN + Doxorubicin treated animals did not showed any significant alteration (Table 12).

Histopathology analysis

Microscopic evaluation of hepatic tissue (a) control showing normal hepatocytes, (b) diethyl nitrosamine (DEN) treated group showing high accumulation of fat with in the hepatocytes, (c-e) DEN + EESG 100, 200, 400 mg/kg treated group and (f) DEN + Doxorubicin 20 mg/kg treated groups showing recovery of normal hepatocytes (H and E, ×400). FA = Fat accumulation, BC = Binucleated cells

DISCUSSION

In Indian system of medicine, certain herbs are claimed to provide relief against liver disorders. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents.[13] One of the most versatile plant used is Sesbania grandiflora, was taken for the anticancer evaluation in DEN induced rat. Elevation of the plasma levels of cytoplasmic and mitochondrial enzymes is a sensitive indicator of liver damage. Excessive antioxidants could dangerously interfere with these protective functions while temporary depletion of antioxidants can enhance anticancer effects of apoptosis

Hepatocellular carcinoma, a highly malignant tumor with extremely poor prognosis, represents 4% of all malignant tumor and is the seventh most common cancer in man worldwide[14] There is now agreement among oncologists that the incidence of cancer is determined by the factors in the environment and it is surprising that diet is suggested to be responsible for about 30–70% of the causes of cancers.[14]

Diethyl nitrosamine is one of the most important environmental carcinogens that primarily induce tumors in the liver, because of its relatively simple metabolic pathway and potent carcinogenic activity.[15] In experimental liver carcinogenesis, early preneoplastic foci appear which are induced by an initiating carcinogen DEN, and they exhibit moderately elevated rate of proliferation.[16,17] Diethylinitrosamine is a powerful hepatocarcinogen known to induce cancer in experimental animals.[18,19] The liver weight increased nearly two folds in those animals that received DEN. The liver weight of animals treated with DEN increased up to 90% and administration of Ethanolic extract of Indigofera aspalathoides (EIA) brought down to 35% compared to control animals[20] whereas, in the present study, the liver weight increased drastically. The weight of the liver was increased to 41%, 17%, 13% in DEN, DEN + EESG, DEN + doxorubicin respectively when compared with control.

Table 1. Effect of ethanolic extract of Sesbania grandiflora on liver enzyme parameters

Group	Treatment	SGPT	SGOT
I	Normal	0.096±0.005	0.136±0.062
II	DEN Control	0.196±0.01*	0.594±0.024*
III	Doxorubicin 20 mg/kg	0.102±0.034***	0.152±0.02***
IV	EESG 100 mg/kg	0.176±0.008***	0397±0.05***
V	EESG 200 mg/kg	0.142±0.003***	0.346±0.057***
VI	EESG 400 mg/kg	0.118±0.004***	0.387±0.051***

Values are expressed as Mean ± S.E.M. number of rats=6.*P≤0.01 Compared with G-1;***P≤0.001 compared with G-2.

Group1: Normal treated rats

Group2: Cancer control rats received Diethyl nitrosamine 20 mg/kg body weight in 0.9% w/v NaCl and was administered intraperitonially

Group 3: cancer rats given doxorubicin (200 µg/kg, i.p.,)

Group 4: Cancer rats given ethanolic extract of *Sespania grandiflora* (EESG) 100 mg/kg b.w.p.o.

Group 5: Cancer rats given ethanolic extract of *Sespania grandiflora* (EESG) 200 mg/kg b.w.p.o.

Group 6: Caner rats given ethanolic extract of *Sespania grandiflora* (EESG) 400 mg/kg b.w.p.o.

Table 2. Effect of ethanolic extract of Sesbania grandiflora on antioxidant parameters

Group	Treatment	GPx	GST	Catalase
I	Normal	14.623±0.26	136±2.62	15.6±2.62
II	DEN Control	18.32±0.1*	594±2.4*	459±2.4*
III	Doxorubicin 20 mg/kg	14.35±0.34***	152±2.9***	251±2.9***
IV	EESG 100 mg/kg	17.6±0.8***	397±5.4***	324±5.4***
V	EESG 200 mg/kg	14.2±0.3***	346±5.7***	316±5.7***
VI	EESG 400 mg/kg	14.8±0.4***	387±5.1***	287±5.1***

Values are expressed as Mean ± S.E.M. number of rats=6.*P≤0.01 Compared with G-1;***P≤0.001 compared with G-2.

Group1: Normal treated rats

Group2: Cancer control rats received Diethyl nitrosamine 20 mg/kg body weight in 0.9% w/v NaCl and was administered intraperitonially

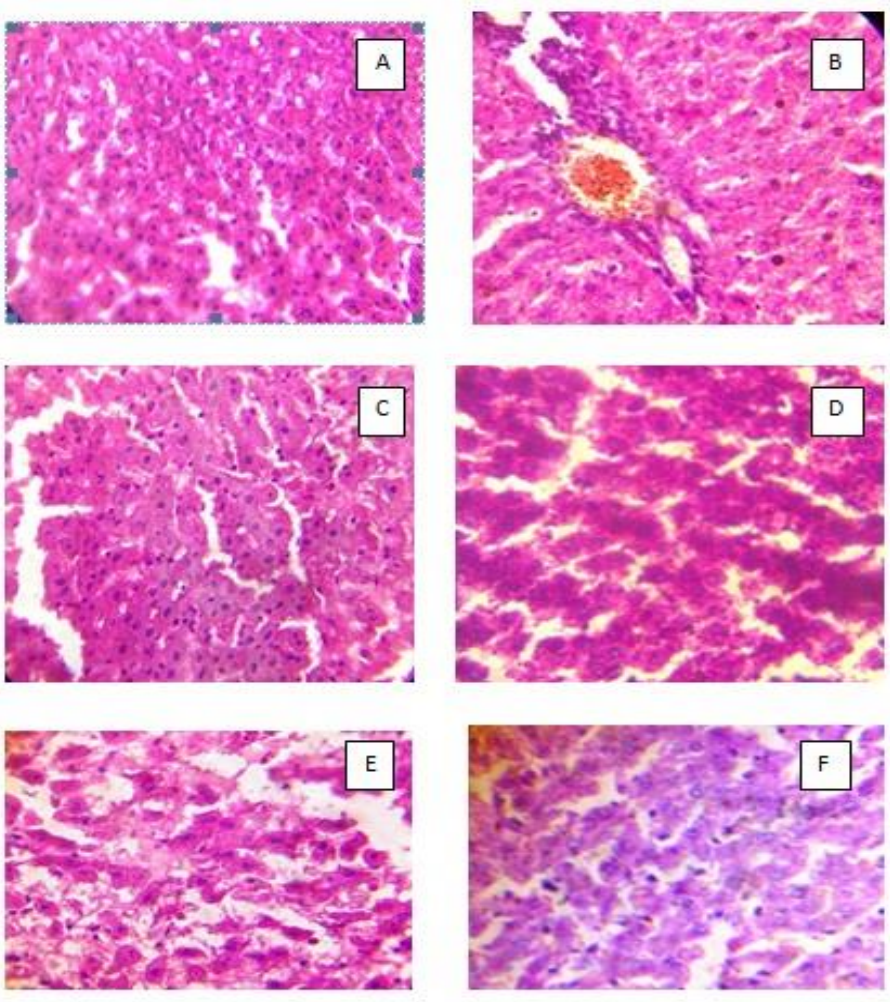
Group 3: cancer rats given doxorubicin (200 µg/kg, i.p.,)

Group 4: Cancer rats given ethanolic extract of *Sespania grandiflora* (EESG) 100 mg/kg b.w.p.o.

Group 5: Cancer rats given ethanolic extract of *Sespania grandiflora* (EESG) 200 mg/kg b.w.p.o.

Group 6: Caner rats given ethanolic extract of *Sespania grandiflora* (EESG) 400 mg/kg b.w.p.o.

Figure 1. Histopathology report



When compared to all groups the DEN treated rat gained increase in body weight that may be due to high metabolic activity of liver and increased accumulation of high-density lipoproteins. The food and water intake was increased in DEN induced rat when compared to control. While in DEN induced group food and water intake slightly increased when compared to DEN + EESG and DEN + Doxorubicin. The effect of EESG is perhaps owing to the abundant of flavonoids presence in it. However, the role of flavonoids is not only limited to radical scavenging activity it may also involve in apoptosis introduction.

In this study, SGOT and SGPT values were decreased in test group when compared to Doxorubicin and DEN treated groups. This is due to high cancerous cell present in the DEN treated groups and thereby high liver marker enzyme synthesis. These results coincide with the results obtained by Jahan et al.[21]

A high level of GST occurs in neoplastic and preneoplastic lesions induced by hepatic chemical carcinogens. The low level of GST in animals receiving the extract + DEN indicates the ability of the EIA to inhibit tumor progression.[22,23] In our result, increased level of GSH has been observed in DEN treated rat. The obtained results in the current investigation concerning GPx and GST enzyme activities were increased in the (test) C. dactylon treated groups when compared to doxorubicin (positive control) treatment. When compared to an earlier study,[22,23] GST shows low significance in DEN and did not show any alteration in saline, DEN + EESG and DEN + Doxorubicin treated groups. GPx shows high significance in DEN, low depletion in DEN + EESG and

did not show any alteration in saline and DEN + Doxorubicin treated groups. DEN induced group may have increased level of lipid peroxidation, that is confirmed by high level of GST and GPx.

Catalase activity in the liver of some transplantable hepatoma cells and cultured hepatoma cell lines have found to be decreased due to the depression of enzyme biosynthesis, which in turn is because of depression of CAT gene expression.[24-26] When Compared with the above study CAT activity in DEN, DEN + EESG showed low significance when compared to control group, whereas DEN + Doxorubicin group did not show any alteration.

The histochemical results of this study revealed the disarrangement of normal hepatic cells with intense centrilobular necrosis in DEN intoxicated liver. A global hepato-protective effect of chrysin administration was evidenced by a marked reduction in vacuolization and disappearance of binucleated cells in animals post treated with oral dose of chrysin. The protective effect of chrysin produced significant decrease in GST-Pi positive foci and PCNA staining in the neoplastic nodules. GST-Pi positive cells are considered precursors of preneoplastic foci that frequently occur during early stages of experimental carcinogenesis.[27] Our present histopathology results showed normal hepatocytes in control animals, whereas DEN induced group showed hepatocytes with hyperchromatic nuclei, prominent nucleoli and marked fat accumulation within the liver cells. In test group animals, proper arrangement of normal hepatic cells was observed.

CONCLUSION

The present study concludes EESG treatment was observed to exhibit anti cancerous effect as demonstrated by enhanced activities of antioxidant enzymes (AST, ALT, GST, GPx and CAT). It may be due to the presence of antioxidant property. This gives an opportunity to find out the active compound which is present in Sesbania grantiflora responsible for the anticancer activity.

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