ANTITUMOR ACTIVITY AND IN VIVO ANTIOXIDANT STATUS OF *T. PURPUREA* AGAINST DENA INDUCED HEPATOCELLULAR CARCINOMA IN SWISS ALBINO MICE

GNANARAJA RAJARETHINAM\(^{a1}\), VEERU PRAKASH\(^{b}\) AND MAHENDRA VERMAN\(^{c}\)

\(^{a}\)Department of Biochemistry and Biochemical Engineering, SHIATS (Formally Allahabad Agricultural Institute Allahabad) U.P., India

ABSTRACT

The aim of the present study is to assess the antitumor effect and antioxidant role of *T. purpurea* against Diethylnitrosamine (DENA) induced hepatocellular carcinoma in Swiss albino mice. The effect of methanol extract of *T. purpurea* (METP) was studied by the following parameters: Alpha fetoprotein, alkaline phosphatase Acid phosphatase and lactate dehydrogenase of the host. METP was administered at a 300 and 400 mg/kg BW once a day for 14 days, after 14 day of DENA supplemented with 3% alcohol. Decrease in AFP, ALP, ACP and LDH were observed in METP treated animals when compared to cancerous animals. The study was also extended to estimate the liver biochemical parameters such as LPO, protein, and antioxidant enzymes like SOD and CAT. Treatment with METP decreased the level of lipid peroxidation and increased the levels of superoxide dismutase (SOD) catalase (CAT) and hepatic protein. The results suggest that the methanol extract of *T.purpurea* exhibits significant antitumor and antioxidant effects in DENA induced hepatocellular carcinoma in albino mice.

KEYWORDS: Hepatocellular carcinoma, *T.purpurea*, antioxidant, Diethylnitrosamine and methanol extract

Medicinal plants play a significant role in providing primary health care services to people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. In present decades, there is increasing interest to unlock the secrets of ancient herbal remedies (Izzo and Ernst, 2009). The natural products of plant origin are a rich source of cancer chemotherapy drugs, and exhibited low or almost no toxicity to normal tissues; hence, more attention is being paid to searching for new antitumor agents from natural products (Dai et al., 2011). More than 60% of the anticancer agents used today are derived directly or indirectly from natural sources Cragget al. (1997).

Natural sources of antioxidants can also be used in nutritional or pharmaceutical fields for the prevention of free-radical-mediated diseases Thambiraj, et al. (2012). The antioxidant is show promise in cancer therapy by their palliative action, reducing painful side effects associated with the treatments Kennedy, et al. (2001). The high antioxidant and antimutagenic activity found in some herbal extracts may help to protect against free radical and reduce mutagen formed in the pH of stomach digestion Kruawan, and Kangsadalampai, (2006).

*Tephrosiapurpurea* is a species of flowering plant in the pea family, Fabaceae, which has a pantropical distribution. It is a common wasteland weed. In many parts it is under cultivation as green manure crop. According to Ayurveda, this plant is used in the treatment of leprosy, ulcers, asthma, and tumors, as well as diseases of the liver, spleen, heart, and blood. Based on this evidence we have selected *T. purpurea* for the present study. The aim of the present study is to evaluate the antitumor properties of the methanol extract of *T. purpurea* (METP) against DENA induced cancerous models along with its antioxidant status.

MATERIALS AND METHODS

Plant Materials and Preparation of Plant Extract

The *Tephrosiapurpurea* plant was collected from SHIATS garden in Allahabad District, in the month of December- 2011. The aerial parts of plants were dried under shed at 25° C and the dried basic were made in to coarse powder. Two hundred grams (200g) of the powered plants were extracted with methanol (80%) using “Soxhlet Apparatus” for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Ethical Clearance

Protocol used in this study for the use of mice as an animal model for cancer was approved by the United Institute of Pharmacy Animal Ethical Committee.

Experimental Animals

Male Swiss albino mice of about 8 weeks of age with an average body weight of 24 ± 2 g were used for the experiment. The animals were bred and brought up in our
laboratory facility with 12-h cycles of light and dark at 23°C. They were fed standard laboratory diet and were given sterilized water ad libitum.

**Experimental Design**

Body weights of the animals were recorded and they were divided into 4 groups. Each group having 6 animals and the details as follow.

**Group 1**

Normal control mice will be fed with standard diet and served as a control.

**Group 2**

Mice induced with hepatocellular carcinoma by single intra-peritoneal injection of DENA 175 mg /kg BW with 3% alcohol for a 14days orally.

**Group 3**

Mice induced with hepatocellular carcinoma mice with methanolic extract of *Tephrosia purpurea* oral gavage daily at the dose of 300 mg/kg for 14 days.

**Group 4**

Mice induced with hepatocellular carcinoma mice with methanolic extract of *Tephrosia purpurea* oral gavage daily at the dose of 400 mg/kg for 14 days.

**Collection of Sample for Biochemical Assay**

On completion of the experimental period, animals were anaesthetized with diethyl ether (2ml/kg). The blood was collected without anticoagulant and serum were separated for the estimation of various biochemical parameters such as Alpha fetoprotein by *Johnson*, (2001); Alkaline phosphatase by *King and Armstrong*, (1934); Acid phosphatase by *Gutman and Gutman*, (1938); Lactate Dehydrogenase (LDH) by *King*, (1965).

**Estimation of in vivo Antioxidants**

After collecting the blood samples, the mice were killed by cervical dislocation. The liver was excised, rinsed in ice-cold normal saline solution followed by cold 0.15 M Tris-HCl (pH 7.4), blotted dried and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-HCl buffer and was used for the estimation of lipid peroxidation (LPO) by *Devasagayam and Tarachand*, (1987) and protein *Lowry et al.*, (1951). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD) by *Marklund and Marklund*, (1997) and catalase (CAT) activity *Sinha*, (1972).

**RESULTS**

In ethanol + DEN treated mice (Group II) caused a significant increase 431.41% in AFP, 153.37% in ALP, 38.25% in ACP and 54.44% in LDH level of serum as compared to normal control mice (Group I) while these were significantly reduced to near normal value in supplementation of methanolic extract of *T. purpurea* @ 300 and 400 mg/kg BW. The maximum decrement of biochemical clinical markers activity of cancerous mice was noted at treatment of plant extract @ 400 mg/kg BW and the minimum decrement was seen at 300 mg/kg BW (table 4.1; fig 4.1).

| Table 1: Antitumor activity of methanol extract of *T. purpurea* (METP) on clinical markers |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Treatment | AFP (ng/ml) | ALP IU/L | ACP (U/L) | LDH (U/L) |
| Normal control | 12.67±2.66 | 74.33±1.21 | 6.85±0.33 | 144.8±6.21 |
| Cancer control | 67.83±2.56 | 188.3±3.50 | 9.47±0.26 | 223.67±3.67 |
| DENA+ METP (300mg/kg) | 55.50±1.87 | 146.0±4.52 | 8.13±0.09 | 177.33±3.93 |
| DENA+ METP (400mg/kg) | 43.33±1.97 | 135.50±4.37 | 7.88±0.25 | 156.33±2.94 |

**Antitumor Effect of METP on Hepatic protein Levels**

There was quite difference in the level of liver tissue protein of experimental mice after 14 days of observation. In normal control control mice, liver tissue homogenate protein level was noted 226.50±5.79 mg/ml respectively. The recovery of protein content in the liver tissue of DENA induced cancerous mice were observed on the feeding animals with two different doses of plant extract and were recorded maximum recovery from 179.50±3.98 mg/ml which was accounted about 16.25% in highest dose i.e. 400 mg/kg BW. The increased protein level in hepatic tissue is to make up the demand of regular metabolism. The lower dose i.e. 300 mg/kg BW of *T. purpurea* extract raised only respectively 6.78% hepatic protein in cancerous mice (table4.2; fig4.3).
Antitumor Effect of METP on LPO activity

In this study, malondialdehyde (MDA) levels are a direct indicator of lipid peroxidation and they were observed to be significantly decreased after DENA treatment (P < 0.005). The control treatment of normal mice did not indicate any changes in MDA levels. The supplementation of plant extracts in 300 mg/kg and 400 mg/kg BW to the DENA induced cancerous mice displayed significant reduction in LPO activity and maximum decrease in activity was detected at 400 mg/kg BW plant extract. The percent decrement of activity at 400 mg/kg BW was recorded 34.69% and followed by 300 mg/kg BW (28.42%) (table 4.2; fig 4.2).

Antitumor Effect of METP on SOD activity

The liver tissue of DENA induced cancerous mice displayed three folds lowers (0.49±0.02 units/min/mg of protein) SOD activity than its normal control. The activity of liver tissue SOD was found to be increased in cancerous mice during two weeks of experimental trial with the administration of two different dosages of plant extracts but percentage of improvement was accorded much more in the liver of mice at all levels of extracts. The maximum percent increment was recorded at the highest dose i.e. 400 mg/kg BW of plant extract of cancerous mice. The percent increase in the activity of SOD due to the supplementation of plant extract @ 300 and 400 mg/kg BW was found to be respectively 77.55% and 95.92% in cancerous mice (table 4.2; fig 4.3).

Antitumor Effect of METP on CAT activity

Liver homogenate entails more attention for observation of enzymatic antioxidant than other tissue in vivo since this effect more rapidly toward oxidative stress due to generation of ROS. Catalase activity in the hepatic tissue of normal control mice was observed to be 69.67±1.12 μ Moles of H₂O₂ consumed/min/ mg protein. The level of hepatic tissue CAT activity was found to be increased by 29.14% respectively in DENA induced normal mice and the mean value was examined to be 49.37±0.79 μ Moles of H₂O₂ consumed/min/ mg protein. The decrease in Hepatic CAT activities of cancerous mice with the supplementation of methanolic extract of T. purpurea was examined. Increasing dosages of plant extracts significantly decreased the activity and maximum decrement in activity was noted 19.55% at highest dose i.e. 400 mg/kg followed by 300 mg/kg BW (11.69%) (table 4.2; fig 4.3).

Table 2: Antitumor activity of methanol extract of T.purpurea (METP) on antioxidant status

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/ml)</th>
<th>Lipid peroxidase (µ Moles of MDA/min/mg protein)</th>
<th>Superoxide dismutase (units/min/mg of protein)</th>
<th>catalase (µ Moles of H₂O₂ consumed/min/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>226.50±5.79</td>
<td>6.75±0.19</td>
<td>1.58±0.06</td>
<td>69.67±1.12</td>
</tr>
<tr>
<td>Cancer control</td>
<td>179.50±3.98</td>
<td>11.33±0.2</td>
<td>0.49±0.02</td>
<td>49.37±0.79</td>
</tr>
<tr>
<td>DENA+ METP (300mg/kg)</td>
<td>191.67±4.63</td>
<td>8.11±0.37</td>
<td>0.87±0.06</td>
<td>55.14±1.21</td>
</tr>
<tr>
<td>DENA+ METP (400mg/kg)</td>
<td>208.67±2.16</td>
<td>7.40±0.21</td>
<td>0.96±0.15</td>
<td>59.02±0.86</td>
</tr>
</tbody>
</table>

Figure 1: Percentage decrease in serum of clinical marker of treated mice compared with cancer controls
DISCUSSION

Diethylnitrosamine (DENA) is reported to be a hepatotoxin and hepatocarcinogenic agent. In the present study, DENA induced hepatocellular carcinoma is clearly evidenced by the marked elevation in serum AFP, ALP, ACP and LDH activity. These biochemical marker enzymes are indicators of tumor response (Thirunavukkarasu and Sakthisekaran, 2003; Narsimha, et al., 2011). In 1994, Kobayashi and Kawakubo reported that the rise in a activity of liver enzymes in cancer bearing animals may be due to the disturbance in the secretory activity or in transport of metabolites or may be due to altered synthesis of certain enzymes in these conditions (Kobayashi and Kawakubo, 1994). Increased transaminase activities in hepato-cellular carcinoma have been reported by Rocchiet al. (1997) which indicate tumor progression, increased DNA synthesis and cell replication in an advanced cancer condition. Present study exposed significant decrease of elevated level of liver functions markers in DENA induced cancerous mice by methanolic extracts of T. purpurea due to possessing important phytochemicals mainly phenols and flavonoids that inhibit the activities of DNA synthesis & cell replication and repaired secretory and transport in liver to blood. The lowering in the activities of AFP, ALP, ACP and LDH on T. purpurea treated mice shows hepatoprotective effect and inhibition of carcinogenesis in liver.

Lipid peroxidase is considered as one of the basic mechanisms of cellular damage caused by free radicals. An increase in lipid peroxides indicates serious damage to cell membranes, inhibition of several important enzymes, reduced cellular function, and cell death (Bananaket al., 2004). These products can attack cellular targets including DNA, thereby inducing mutagenicity and carcinogenicity (Zwartet al., 1999). Lipid
peroxidation and associated membrane damage are key features of DEN-induced carcinogenesis (Aniset al., 2001). The increased LPO level confirms the cancerous condition in DENA induced hepatocellular carcinoma animals (Banakaret al., 2004). However, the administration of T. purpurea methanolic extract decreased the LPO levels in DENA treated cancerous mice which may be due to the free radical scavenging activity of the T. purpurea methanolic extract.

SOD is said to act as the first line of defense against superoxide radical generated as a by-product of oxidative phosphorylation (Sallie et al., 1991). The malignant cells of different cancer types exhibit heterogeneity in the levels of oxidative stress, associated with various expression levels of SOD and other antioxidant enzymes. Decreased SOD activity was observed in various cancerous conditions (Selvendiranet al., 2003) and diminished scavenging of free radicals formed in liver cancer conditions. Thus our result put forward a valuable property of T. purpurea methanolic extract in curing SOD activity in cancerous condition. In this context other work also reported similar effects with different plant extracts that support our observation.

In our studies, catalase activity decreased significantly upon DENA induced oxidative stress in cancer animals and the effect being moderate in groups treated with plant extract. Decreased catalase activity indicates that DENA induced oxidative stress interferes with the anti-oxygenic potential in the tissues, thus resulting in increased generation of ROS (Bartschet al. 1989; Bansalet al. 2005). The existence of a mechanism for the movement of catalase across cell membranes would explain the reason for the decrease in liver catalase activity in cancerous conditions (Cranet al., 1982). Present experimental findings reveal increase in CAT activity in cancerous mice induced by DENA after treatment with methanolic extract of T. purpurea.

In conclusion, the present study supplementation of methanol extract of T. purpurea (METP) decreased in elevated level of AFP, ALP, ACP, LDH and Lipid peroxidase activity as well as increased in reduced SOD and CAT activity in hepatocellular mice. All these parameters suggest that the methanol extract of T. purpurea exhibits potential antitumor and antioxidant activities against DENA induced cancerous mice.

CONCLUSION

All these observations clearly indicate a significant anticancer and antioxidant effect of the methanolic extract of T. purpurea. Further studies to characterize the active principle and to elucidate the mechanism of action are in progress.

ACKNOWLEDGEMENT

The authors acknowledge the SHAITS, Deemed to be University for providing lab facilities and also thankful to Dr. J. Androw and Dr. Alok Mukerjee, provision to complete this work.

REFERENCES


