International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 565-568

Received: 29-07-2017
Accepted: 30-08-2017

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Hepatoprotective effect of *Cleome gynandra* on Ehrlich Ascites Carcinoma in mice

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Abstract
Cleome gynandra has been widely used for many ailments in Ayurveda and Siddha system. In the present study the hepatoprotective effect of 70% ethanolic extract of *Cleome gynandra* (EECG) was investigated against Ehrlich Ascites Carcinoma (EAC) mice. Various antioxidant parameters like reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), lipid peroxidase (LPO) and catalase (CAT) were studied in the present communication. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Treatment with EECG at 300 mg kg^{-1} for 10 days followed by tumor inoculation significantly reduced the impact of EAC on hepatocytes. The histopathology studies also supported that *Cleome gynandra* markedly reduced the effect of EAC on liver tissue near to normal. Thus the study reveals the potent hepatoprotective property of *Cleome gynandra*.

Keywords: *Cleome gynandra*, EAC, Antioxidant parameters, Hepatopotective property.

Introduction
Free radical is the major element behind the conversion of normal cells to cancerous cells, which will be generated by a number of endogenous metabolic processes involving redox enzymes, bioenergetic electron transfer and exposure to multitude of exogenous chemicals. Erratic production of free radical and reactive oxygen species would assault on important biological molecules such as DNA, protein or lipid leading to degenerative diseases like cancer. Natural Products, specifically plants, have been used for the treatment of various diseases from ancient times. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them. The first written records of the medicinal uses of plants appeared in about 2600 BC [1]. Generally, phytochemicals exert their antiproliferative role by modulating the apoptotic signalling pathways through antioxidants or some unexplored mechanism in cancer cells which is considered as the key event in the antitumor activity [2].
The best approach in the search of antitumoral activity of natural products is to prefer plants based on its traditional and ethno-medicinal usage. In this perspective, *Cleome gynandra* Linn. is brought to light.
Cleome gynandra Linn. of Capparidaceae family is an indigenous medicine reported to have rubefacient, vesicant, antiseptic, anti-inflammatory, anthelminthic and analgesic properties. The extract of this plant also possesses anticancer, antibacterial, antimycotic and antioxidant properties [3].
The present study is to validate the antitumorgenic property of *Cleome gynandra*, through assessment of different parameters and as EAC was expected to affect liver cells the liver antioxidant parameters are of paramount importance.

Materials and Methods
Drugs and chemicals
The pure salt of 5-Fluorouracil (5-FU) was obtained from Sigma Aldrich (USA). All other chemicals and solvents used throughout the study period were of analytical grade.

Collection of plant material
The whole plant of *Cleome gynandra* Linn. was collected (about 10kg) from the Kancheepuram District, Tamil Nadu, India. The specimen was authenticated at Department of Botany, Presidency College, Chennai, Tamil Nadu.

Extraction procedure
The collected plant materials were shade dried for a month without any contamination and then coarsely pulverized. The powders were then packed into Soxhlet apparatus and subjected to continuous hot percolation at 70 °C using 70% ethanol as solvent for about 12 to 15 h (30-35 cycles) until the solution appears clear [4]. The extracts obtained were then concentrated under reduced pressure under vacuum in rotary evaporator (Heidolph, India) at 40°C and 30 rpm and suspended in double distilled water for further antitumor studies.

Qualitative phytochemical analysis
The 70% ethanolic extract of *Cleome gynandra* was screened for their phytochemical constituents according to the standard methods as discussed in previous literatures [5, 6].

Experimental animals
Swiss albino mice of either sex, aged about 6-8 weeks weighing approximately 25 g, procured in 36 numbers from Laboratory Animal Medicine Unit, Tamil Nadu Veterinary and Animal Sciences University, Chennai, were used for this study. They were housed in sterile polypropylene cages, as 3 mice per cage and maintained under standard laboratory condition. They were fed with standard laboratory animal pellet feed and water *ad libitum*. They were allowed to acclimatize to the environment for 7 days prior to experimental period. The experiments were performed in accordance with the guidelines of CPCSEA after getting clearance from the Institutional Animal Ethical Committee (IAEC), Chennai, India (Proposal No: 2345/22/DFBS/IAEC/2016).

Acute oral toxicity study
The acute oral toxicity studies for the 70% ethanolic extract of *Cleome gynandra* Linn. was conducted in accordance with the up-and-down-procedure of Organization for Economic Cooperation and Development (OECD) guidelines 425 [7]. The animals received 70% ethanolic extract of the plant suspended in double distilled water, starting at 2 g kg^{-1} orally by gavage and were observed individually for aberrations in the cardinal signs, toxic symptoms and mortality periodically until 14^{th} day. Finally the number of survivors were noted, checked for any gross lesions and then sacrificed to look for any damages to the internal organs.

EAC cells
EAC cells were obtained from Amala Cancer Research Centre, Kerala, India and they were maintained under our laboratory conditions *in vivo* in female Swiss Albino mice.

Transplantation of tumor
Ascitic fluid was collected from the EAC bearing animals at the log phase (days 7-8 of tumor inoculation) and the cells were washed with PBS. The cell count was adjusted so that the cell concentration was 5×10^6 cells in 0.2 mL of PBS. Then, each animal of the experimental unit received 0.2 mL of tumor cell suspension intra-peritoneally.

Experimental design
The animals were randomly divided into six groups with each group containing 6 mice.
Group-1 Normal healthy control: The animals were not inoculated with EAC cells.
Group-2 Tumor control: The animals were inoculated with EAC cells, received only distilled water for 10 days (Tumor model).
Group-3 Standard drug (5-FU) treatment group: EAC inoculated and treated with 5-FU (20 mg kg^{-1} orally from 24 h of inoculation to 11th day).
Group-4 EECG 300 mg kg-1 treatment group: EAC inoculated and treated with 70% ethanolic extract of Cleome gynandra @ 300 mg kg-1 (orally from 24 h of inoculation to 11th day).
Group-5 EECG 150 mg kg-1 treatment group: EAC inoculated and treated with 70% ethanolic extract of Cleome gynandra @ 150 mg kg-1 (orally from 24 h of inoculation to 11th day).
Group-6 EECG 75 mg kg-1 treatment group: EAC inoculated and treated with 70% ethanolic extract of Cleome gynandra @ 75 mg kg-1 (orally from 24 h of inoculation to 11th day).

Treatment schedule and sacrifice
Treatments with 5-FU (20 mg kg^{-1}.) and the 70% ethanolic extract of *Cleome gynandra* were started from the 24^{th} hour of tumor inoculation and were then continued for 10 days. At the end of the treatment, mice were sacrificed and dissected; liver was collected for histopathology in formalin and a part was stored in -20 °C which was later homogenized within 12 hours and used for estimation of antioxidant levels.

Histopathological studies
Liver specimen obtained from the control and treated mice were fixed in 10% buffered formalin. The formalin fixed liver samples were processed for tissue sectioning and stained with haemotoxylin-eosin to observe the histopathological architecture.

Assessment of liver antioxidants parameters.
The liver from each mouse was excised and rinsed in ice cold normal saline. Within 3 hours of sacrifice, 10% w/v liver homogenate was prepared in ice cold Tris-HCl (0.1M, pH 7.4) buffer and made into several aliquots for estimation of glutathione peroxidase (GPx), lipid peroxidase (LPO), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH).

Statistical analysis
Results were analyzed by complete randomized design using SPSS® software (version-20) and comparison of the means was done by using Duncan's Post-hoc test (multiple comparison tests). The results were expressed as mean ± S.E.

Results
Qualitative phytochemical analysis
The 70% ethanolic extract of the *Cleome gynandra* was qualitatively analysed for the presence of different phytochemical constituents and detected to contain alkaloids, flavonoids, terpenoids, phenols, saponins, quinone, glycosides and tannins. However, steroids, anthacyanin / betacyanin and proteins were absent in the extract.

Acute toxicity
The 70% ethanolic extract of the *Cleome gynandra* was found to be safe since no observed derangements in the animals was noticed up to 14 days at a maximum dose of 2000 mg kg^{-1}. Hence, the plant extract was not acutely toxic when administered orally up to the level of 2000 mg kg^{-1}.

The effects of the plant extract on Liver antioxidant parameters

Reduced glutathione (GSH), Catalase (CAT) and Superoxide dismutase (SOD) was decreased significantly ($P<0.05$) in tumor control mice compared to that of normal control animals. The values were increased in the treatment groups. Treatment with extract of *Cleome gynandra*, increased the level of GSH, CAT and SOD which differed significantly ($P<0.05$) from that of tumor control animals. Similar results are seen upon treatment with the standard drug 5-FU. It shows a dose dependant increase in efficacy (Table-1). The level of Glutathione peroxidase (GPx) and Lipid peroxidase (LPO) was noticed to be elevated significantly ($P<0.05$) in tumor control mice compared to normal control animals. The values decreased in the treatment groups. Treatment with 5-FU and 70% ethanolic extract of *Cleome gynandra*, effectively decreased the level of GPx and LPO which differed significantly ($P<0.05$) from that of tumor control animals. The effect of plant extract has significantly similar effect to that of 5-FU except at high dose of plant extract (300 mg kg^{-1}). The results are presented in Table-1.

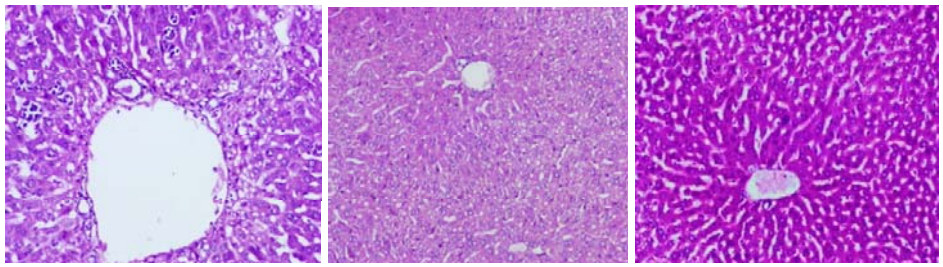
Table 1: Effect of plant extract on liver antioxidant parameters

Groups	GSH (nm/mg)	GPx (Units/mg)	LPO (nm/mg)	SOD (Units/mg)	CAT (Units/mg)
Normal control	$24.48^{a} \pm 0.8$	$65.20^{a} \pm 1.38$	$9.83^{a} \pm 1.03$	$9.03^{a} \pm 0.06$	$26.00^{a} \pm 0.28$
Tumor control	$12.26^{e} \pm 0.35$	$98.00^{e} \pm 1.93$	$30.36^{d} \pm 1.15$	$3.25^{d} \pm 0.02$	$10.66^{f} \pm 0.43$
5-FU treatment	$26.23^{a} \pm 0.72$	$72.93^{b} \pm 0.52$	$13.75^{b} \pm 0.54$	$8.20^{ab} \pm 1.05$	$23.07^{b} \pm 0.40$
EECG-75 mg kg^{-1}	$16.48^{d} \pm 0.74$	$85.54^{d} \pm 0.78$	$17.97^{c} \pm 1.26$	$5.60^{c} \pm 0.10$	$16.23^{e} \pm 0.25$
EECG-150 mg kg^{-1}	$19.08^{c} \pm 0.67$	$79.43^{c} \pm 2.42$	$15.19^{bc} \pm 0.60$	$7.16^{b} \pm 1.15$	$18.50^{d} \pm 0.51$
EECG-300 mg kg^{-1}	$21.73^{b} \pm 0.98$	$76.35^{bc} \pm 1.99$	$14.30^{b} \pm 0.47$	$7.76^{ab} \pm 1.00$	$21.49^{c} \pm 0.82$

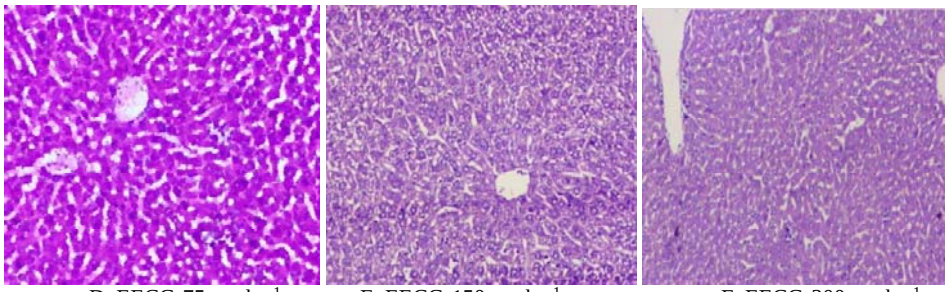
Means bearing different superscripts in a column differ significantly between groups ($P<0.05$).

The effect of plant extract on histopathology of liver

The hepatoprotective effect of 70% ethanolic extract of *Cleome gynandra* was confirmed by histopathological examination of the liver tissue collected from the control and treated mice. The tumor control liver section showed multifocal severe mononuclear cell infiltration, while in normal control animals liver architecture was found to be normal. Treatment with plant revealed a sign of protection as it evident with moderate to mild mononuclear cell infiltration. The figures are presented in Figure-1.



A. Tumor Control B. Normal Control C. 5-Fu Treatment



D. EECG-75 mg kg^{-1} E. EECG-150 mg kg^{-1} F. EECG-300 mg kg^{-1}

Discussions

In the present study, during the analysis of liver biochemical parameters it was found that the levels of lipid peroxidation and glutathione peroxidase were significantly increased in tumor control mice compared to normal healthy control animals which is in accordance to previous findings [8, 9], where it was stated that tumor cells tend to produce more peroxides, when they proliferate actively after inoculation of tumor and known to affect functions of many vital organs, which indicated the intensification of oxygen free radical production. However, the levels of the other enzymatic and non-enzymatic antioxidants like GSH, SOD and CAT, were significantly decreased in tumor control animals compared to normal control group which act as the markers of malignant transformation [8]. Treatment with the standard drug in our study significantly retracted those deranged values towards that of the normal healthy control animals which are in accordance with [10]. The plant extracts acted almost in a similar fashion to that of the standard drug, whereas, the significant effects were noticed at 150 and 300 mg kg^{-1} of the plant extract over lipid peroxidation and superoxide dismutase and 300 mg kg^{-1} dose level exhibited significant effects in all the values except GSH and catalase. Thus, the plant extracts may be able to do so by protecting the hepatocytes by reducing the oxidative stress induced by tumor. EAC induces damage to hepatocytes by metastasis and invasion [11], and the early signs are the appearance of inflammatory cells in multifocal fashion which increases with tumor progression

ranging from mild to severe. Genetic alterations in cancerous cells promote a continuous and elevated production of Reactive oxygen species (ROS). Although such oxidative stress conditions would be harmful to normal cells, they facilitate tumor growth in several ways by causing DNA damage and genomic instability, and eventually, by reprogramming cancer cell metabolism, which may be by oncogene activation and down regulation of tumor suppressor genes [12]. The treatment with 70% ethanolic extract of *Cleome gynandra* was able to decrease the progression of tumour hence protecting the hepatocytes which was noticed in our study similar findings are also noticed in methanolic extract of *Cleome gynandra* on Carbon tetrachloride induced hepatotoxicity in Wistar rats [13]. The effect may be due to antioxidant properties of phytochemicals present in the extract which are able to alleviate the level of free ROS produced by cancerous cells. The cytotoxic effects of polyphenols and flavonoids have been discussed earlier where, it has been stated that flavonoids like rutin and quercetin have been shown to possess antimutagenic and antitumor effects [14, 15]. Also, quercetin has been proved to inhibit human breast cancer cells and prostate cancer cells [16]. Thus, the effect of our plant extract on supressing the progression of EAC may be due to the presence of flavonoids, phenols and triterpenoids.

Conclusion

It can be conclude that the 70% ethanolic extract of *Cleome gynandra* at 300 mg kg^{-1} possess a protective effect against EAC induced hepatotoxicity in mice as evidenced by the liver antioxidants and histological parameters.

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