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Full Length Original Research Paper

Hepatoprotective activity of *Caesalpinia bonducella* leaves against Carbon Tetrachloride induced Hepatotoxicity

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Abstract: Natural products serve as lead molecules for development of many popular drugs. Herbal drugs are having fewer side effects than the other class of drugs which are coming from the synthetic source. *Caesalpinia bonducella* found throughout the hotter parts of India. Based on literature survey as it showing so many bioactive components and have higher medicinal values with lesser side effects. Hence an attempt was made to screen the hepatoprotective activity of *Caesalpinia bonducella* leaf extracts in carbon tetrachloride induced hepatotoxicity. Histological profile of control animals showed normal hepatocytes. Group II animals exhibited intense centrilobular necrosis, vacuolization and macro vascular fatty change. The section of liver taken from the animals treated with standard drug silymarin showed hepatic architecture, which was similar to that of control. The animals treated with hydro alcoholic exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration. However moderate accumulation of fatty lobules was noted in the section of animals treated with the pet. ether extract.

Keywords: *Caesalpinia bonducella,* Carbon tetrachloride, Histological profile, silymarin.

Introduction

Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. In olden times, vaidyas used to treat patients on individual basis, and prepare drug according to the requirement of the patient. But the science has changed now; herbal medicines are being manufactured on large scale in mechanical units. Alternative approach to the drug discovery is through the medicinal plants. Many numbers of people seeking remedies and health care approaches free from side effects caused by synthetic chemicals recently, attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human disease [1]. The present work based on the fact that *Caesalpinia bonducella* belonging to the family *fabaceae* (*caesalpiniaceae*) found through the hotter part of India and available easily in our

locality, and cultivated largely in the near parts of our district, Kurnool of Andhra Pradesh. Extensive literature survey revealed that, a detailed study was conducted on the Pharmacognostic profile of seeds [2], kernels of seeds [3], stem [4], and leaf [5] of *Caesalpinia bonducella*. *C.bonducella* showing many bioactive components and has higher medicinal values with lesser side effects. Hence, it is worthwhile to carry out a systematic and scientific screening for its hepatoprotective activity.

Materials and methods

Collection and processing of plant material

The leaves of *Caesalpinia bonducella* were collected from local forest area of Kosigi, Kurnool District of Andhra Pradesh, India with the help of tribal people, during in the month of November 2010. The plant material was botanically identified and authenticated by Dr. C. Venkataramaiah, Reader in botany, V R College, Nellore, S.P.S.R. Nellore District of Andhra Pradesh, India and the same has been deposited as a voucher specimen with reference No. VCHS No. 1520 for future reference in the department of botany, VR College, Nellore, S.P.S.R. Nellore District, Andhra Pradesh, India. The leaves were made free from all foreign matter and checked thoroughly for any plant parts. They were washed twice, shade dried and powdered.

Preparation of leaf extract

The powder material was subjected to cold maceration process for 72 hrs using hydro alcohol i.e. water and ethanol in 1:1 ratio, and Petroleum ether. Both the extracts were evaporated to dryness under vacuum. The dried extract was stored in vacuum desiccators [6]. The yield of hydro alcoholic (HACB) and petroleum ether extracts (PECB) were 3.6%w/w and 1.2% w/w respectively.

Acute toxicity study

Acute toxicity study was performed according to OECD guideline No 423. Animals were fasted prior to dosing, food but not water should be withheld overnight. Following the period of fasting, the animals were weighed and extract was administered. Three animals are used for each step. The dose level of hydro alcoholic and petroleum ether extracts to be used as the starting dose is selected from one of the five fixed levels 500, 1000, 1500, 2000 and 2500 mg/kg body weight of both the extracts HACB and PECB. The starting dose level should be that which is most likely to produce mortality in some of the dose animals. After administration of test sample, the animals were observed continuously for first 4 hrs for behavioral changes and at the end of 24 hr for mortality rate if any. The animals do not shown any gross behavioral changes and mortality. Hence, both the extracts were selected for pharmacological screening of hepatoprotective activity at a dose of 250mg/kg body weight.

Hepatoprotective activity

Five groups of animals six in each were used for the study. The animals from Group- I served as the control and received the vehicle 1% w/v gum tragacanth at a dose of 1 ml per kg /day of orally for 14 days. Group II-IV received 0.1 ml per kg /day of CCl_4 for 14 days intra peritonially. The standard drug silymarin was administered to group-III animals in the dose of 100 mg/ kg/ day orally for 14 days. While group IV & group V were treated with hydro alcoholic extract (HACB) and petroleum ether extract (PECB) of *Caesalpinia bonducella* at dose of 250 mg /kg/day orally (as per acute toxicity studies) for 14 days. The carbon

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tetrachloride, silymarin and the extracts were administered concomitantly to the respective group of animals. The experimental protocol was approved by the institutional Animal Ethical Committee of Vagdevi College of Pharmacy And Research Centre, Brahmadevam, Nellore-524346.

Assessment of hepatoprotective activity

All the animals were sacrificed on the day 14 under light ether anaesthesia. The blood samples were collected separately by carotid bleeding into sterilized dried centrifuge tubes and allow to coagulate for 30mins at 37^0 C. the clear serum was separated at 2500 rpm for 10 min and biochemical investigation were carried out to asses liver function which includes total bilirubin [7, 8], total protein [9, 10], serum transaminase [11] and serum alkaline phosphatises [12]. The histopathology was also conducted and all the results were tabulated [13, 14]. The results are expressed as mean ± SEM of six animals each group. The data were evaluated by one-way ANOVA followed by Turkey's multiple comparison tests. p values ≤ 0.01 were considered statistically significant.

Results and discussion

The effect of both the extracts HACB and PECB were calculated and tabulated in table No. 1. For all the parameters that is bilirubin, protein, AST, ALT, and ALP, the effect of hydro alcoholic extract was statistically significant (figure no. 1) in response to standard and control groups ($p<0.001$). From the above findings, it is clear that, the hydro alcoholic extract possessing a marked hepatoprotective action (Table No. 1) in the treated animals. Histological profile of control animals showed normal hepatocytes. Group II animals exhibited intense centrilobular necrosis, vacuolization and macro vascular fatty change. The section of liver taken from the animals treated with standard drug silymarin showed hepatic architecture, which was similar to that of control. The animals treated with hydro alcoholic extract exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration. Hower moderate accumulation of fatty lobules was noted in the section of animals treated with the petroleum ether extract. As there was no significant changes in the petroleum ether extract treated group, it was expressed in the histo pathological study. (Fig No.2)

Conclusion

The Ccl_4 has been used as a tool to induce hepato toxicity in experimental animals. This toxic chemical caused per oxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in trans aminase and alkaline phosphatases was the clear indications of cellular leakage and loss of functional integrity of the cell membrane [12]. The above proceedings are clearly demonstrating that the hydro alcoholic extract is a good herbal hepatoprotective agent. The possible reason for this activity may be the presence of flavonoid and phenolic compounds as secondary metabolites in the leaf extract. If this data is validated in clinical trials, *Caesalpinia bonducella* may offer an effective herbal hepato protective agent.

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Group	Bilirubin	Protein	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	0.49±0.03	9.25±0.12	51.07±0.61	148.90±0.36	173.61±2.57
CCl_4 treated	2.22±0.13	6.09±0.32	1341.3±28.28*	2155.45±42.55	404.10±15.11*
CCl_4 +Silymarin 100 mg/kg	0.50±0.01**	8.72±0.01**	89.04±0.42**	205.05±0.92**	181.67±0.52*
CCl_4+HACB 250mg/kg	0.65±0.04**	8.17±0.03**	145.50±0.62**	225.20±0.86**	192.27±0.72**
CCl_4+ PECB 250mg/kg	0.91±0.05**	7.27±0.05**	187.40±0.97**	282.51±1.02**	217.17±1.32**

All the values were expressed as Mean ± SEM.
*P<0.01 compared to control group, and **P<0.001 in response CCl_4 –treated group

Table No. 1: Effect of *Caesalpinia bonducella* leaf extracts on various biochemical parameters

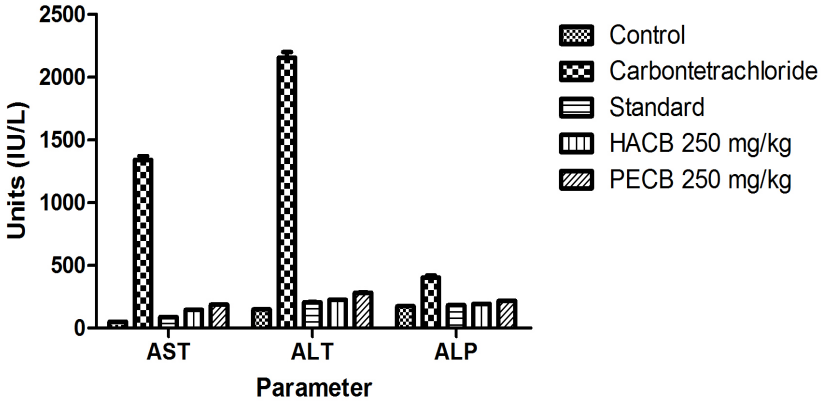

Fig. No. 1 Effect of *C. bonducella* leaf extracts on various biochemical parameters

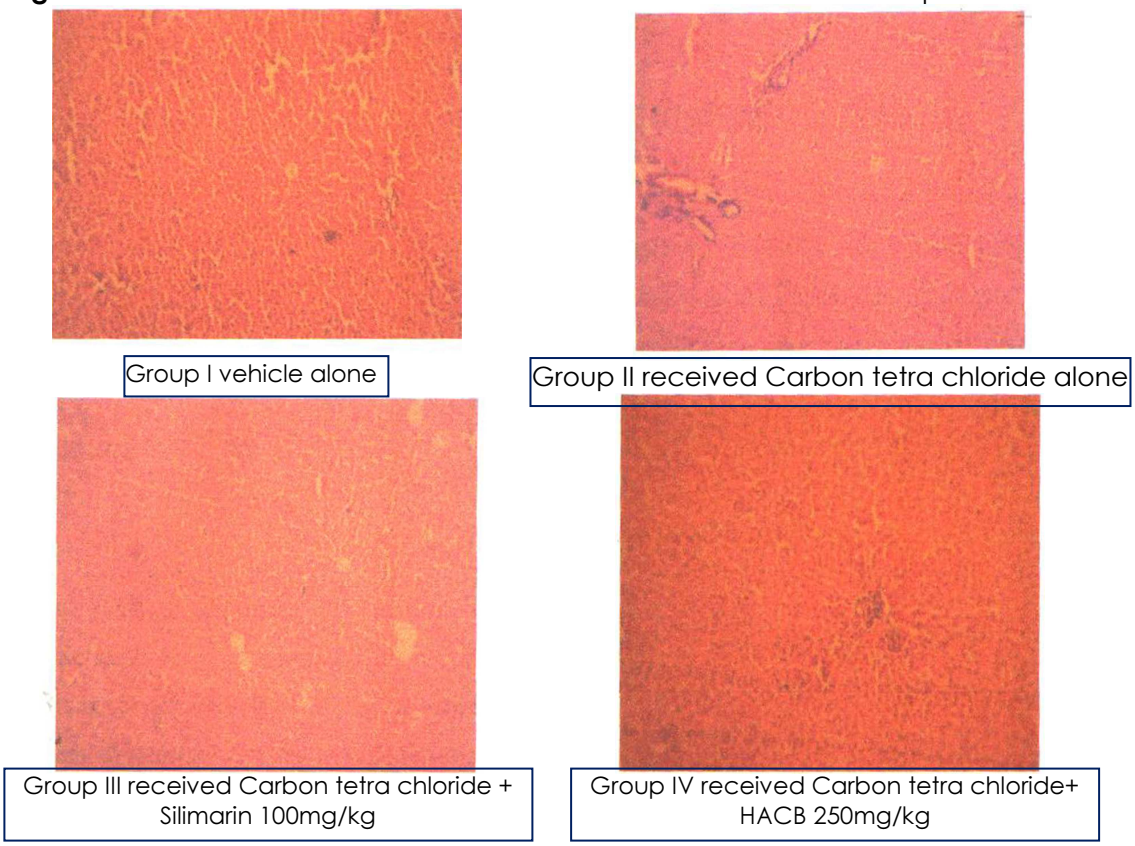

Fig. No. 1 Histopathological characters of different groups treated with control, standard and test extracts

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