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EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF ACACIA CATECHU WILD IN PARACETEMOL INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

Introduction: *Acacia catechu is a deciduous, thorny tree which grows up to 12-15 meter in height. In Hindi is called as Khair and in kannada Kachu. It has been used as used for the treatment of inflammation, diarrhheoa, fever.* ***Aims:*** *to evaluate the hepatoprotective effect of ethanolic extract of acacia catechu in paracetemol induced liver damage in experimental animals, rats was studied.* ***Methodology****: The ethanolic extract of acacia catechu was studied for its hepatoprotective effect on paracetamol induced liver damage on Wistar albino rats. The degree of hepatoprotection was measured by biochemical (serum gultamic pyruvic transaminase, serum gultamic oxaloacetic transaminase, alkaline phosphatase) markers and histological changes. Silymarin was used as positive control.* ***Results****: Pretreatment with acacia extract significantly prevented the biochemical enzyme levels and histological changes induced by paracetamol in the liver. The effects of acacia catechu were comparable to that of the standard drug silymarin. Thus ethanol extract of acacia was found to exhibit marked hepatoprotective activity (P<0.05).* ***Discussion and conclusion:*** *These results indicate that acacia catechu could be useful in preventing chemically induced acute liver injury. Hence from this study it can be concluded that ethanol extract of acacia catechu possess significant hepatoprotective activity.*

KEY WORDS

Acacia catechu, hepatotoxicity, paracetemol, silymarin

INTRODUCTION

Liver disorders constitute serious health problems in India. Currently there are various drugs of allopathic medicine are available which show significant hepatoprotective action, yet plant products/extracts play a vital role in this scenario. India has a rich source of traditional medicines, many of which are of plant origin.

Liver disorders are mainly inflammatory in nature i.e. acute, sub-acute and chronic hepatitis of various aetiologies like viral infection- hepatitis A, B, C, E, chronic alcohol consumption, toxin induced, drug induced and malignancy associated disorders. Liver disorders can vary from asymptomatic elevation of biochemical markers to symptomatic presentation like jaundice, pain abdomen, vomiting, fatigue and even go for severe organ failure and coma [1].

India is being rich source of traditional medicines, many of which are plant origin. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. There are more than one hundred compounds of plant origin, which have shown hepatoprotective potential.

In this study, we evaluated the hepatoprotective action of acacia catechu wild, in paracetemol induced hepatitis in albino rats. Pale catechu or katha (in Hindi) or cutch is an important product of acacia catechu (leguminosae). Catechu the extract prepared from the acacia catechu wood has been used for treating fever, diarrhoea, leucorrhoea, piles, erysipelas. It has also shown hypoglycaemic activity [2].

The juice from the fresh bark has been used in treatment of haemoptysis and gonorrhoea. It also has expectorant activity [3]. Recently its antibacterial action has been evaluated [4].

Catechu contains – catechuic acid, catechu tannic acid (25-33%), acacatechin (10-12%), catechu red, quercetin, catechin (2-12%), epicatechin, phlebotanin (25-33%), gummy matter, quercitrin, quercitin and moisture. Quercitrin is a phenolic flavonoid and catechu of acacia is a pseudotanin. Catechin and epicatechin usually accompany other flavonoids [2,3].

Considering the above facts, the present study was undertaken to evaluate the hepatoprotective property of acacia catechu in experimental animal model i.e. paracetemol induced hepatotoxicity in albino rats**.**

MATERIALS AND METHODS

Collection and Preparation of extract

Catechu (550 gm) was collected from the local market during the month of August 2012and differentiated from uncaria gambier by fluorescin test The plant was identified by botanist and voucher specimen was numbered and deposited in the Herbarium of Department of Pharmacology of S. Nijalingappa medical college, Bagalkot, Karnataka.

The ethyl extract of acacia catechu obtained by making powder of catechu with the help of grinder. Pale coloured powder is obtained, which is deflated with ether to remove the non-polar and phenolic compounds. Then catechu was extracted with 95% ethanol. Then air dried the extract for 48 hours and it was again extracted with ethanol to have concentrated extract.

Acute toxicity Study

The acute toxicity study of heartwood powder of *Acacia catechu* was carried out on Swiss mice with a dose of 2, 4 and 6 g/Kg body weight orally. A single dose of *Acacia catechu* was administered in the form of slurry (water as vehicle).The animals were observed for changes in parameters like body weight, food and water intake and were reported. There is no sign of toxicity. There was no mortality recorded even at the highest dose level of 6gm/kg body weight. [14-16].

Phytochemical analysis

The extract material was subjected for phytochemical analysis. It showed the presence of alkaloids, tannins, proteins, carbohydrates, resins were present.

Study drugs

Paracetemol, Silymarin, acacia catechu extract were used in this study.

METHODOLOGY

Study animals

24 albino wistar rats of either gender weighing 100-150gm were selected from the central animal house of S Nijalingappa Medical College, Bagalkot, Karnataka, India. The experimental protocol was approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government. (**Registration No: 829/AC/04/CPCSEA**). No protocol related procedures were undertaken before taking animal ethics committee approval. All procedures carried out in the study were as per CPCEA guidelines. Selected animals were examined and screened for general health condition including vital parameters. All the animals were acclimatized to laboratory for one week before starting the study. The animals were housed under standard laboratory conditions maintained at 25 ± 5^{o}C and exposed to 12 hr dark and 12 hr light cycle and fed with standard pellet diet and water *ad libitum*.

Induction of hepatotoxicity

These animals were grouped randomly into 4 groups i.e. 6 animals in each group randomly (n=6).

Groups include group –I: Vehicular control group received water (5ml/kg p.o.) for 7 days served as control group

Group-II: Paracetemol group received paracetemol (250mg/kg p.o.) daily for 7 days along with water (5ml/kg p.o.)

Group-III: Silymarin group received standard drug Silymarin (25mg/kg p.o.) daily for 7 days along with paracetemol (250mg/kg p.o.) daily.

Group-IV: Test group received acacia extract (250mg/kg p.o.) daily for 7 days along with paracetemol (250mg/kg p.o.) daily.

On the 7th day of study, blood was drawn from all animals in separate containers for analysis. Laboratory values i.e. SGOT, SGPT and serum alkaline phosphatase (ALP) of all animals is obtained and tabulated.

Histopathological study

Then animals were sacrificed and a portion of hepatic tissue is obtained and washed with normal saline. Then these liver tissues were fixed in 10% buffered neutral formalin for 48 hours. Then thin 5mm sections were made by using microtome and stained with haematoxylin and eosin. Then evaluation was done under light microscope.

Statistical analysis:

Data were analysed using the software SPSS version19.0 to calculate standard deviation, mean and standard error of mean. Comparison between the groups was done using one way ANNOVA with post hoc Turkeys multiple comparison test using SPSS version 19 software. Difference between groups was considered to be significant if Null hypothesis rejected > 95% confidence interval ($p<0.05$).

RESULTS

Table 1 depicts the changes in biochemical parameters in the control and various experimental groups. Group I shows values within physiological range for parameters like SGOT, SGPT and ALP. Whereas there was a significant raise in these values in group II who received paracetemol indicating hepatic injury caused by paracetemol. Group III showing reduction in these values because of the standard control i.e. Silymarin. Group IV which consists of, acacia extract shows decrease in mean values comparable to that of group III.

Table 2 shows comparison among various groups using ANNOVA and post hoc Turkey test.

There was significant difference between control group and paracetamol group showing raise in lab values was statistically significant. Silymarin control group (III) values and acacia extract group (IV) were comparable statistically, there was no significant difference.

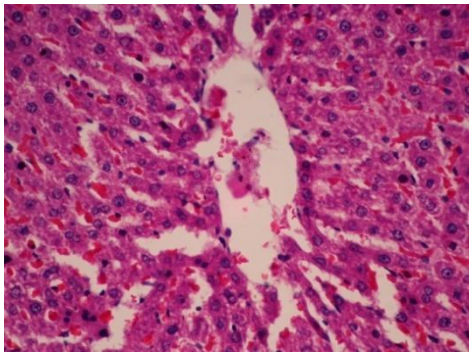
Table: 1 Effect of acacia catechu extract on paracetemol induced hepatotoxicity in rats

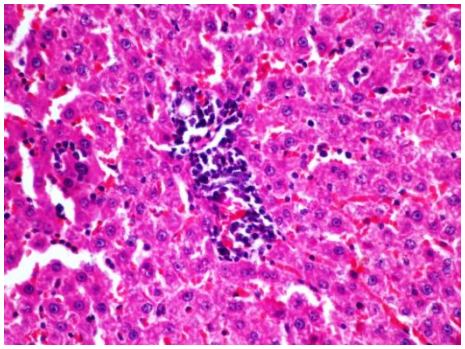
Groups	SGOT	SGPT	ALP
I (Vehicle control)	171.17±01.2	70.92± 00.76	177.85±2.09
II (Paracetemol control)	284.23±11.82	229.57±06.61	202.40±3.18
III (Silymarin +Paracetemol)	222.85±16.38	127.23±15.71	188.30±5.06
IV(Acacia extract + Paracetemol)	223.32±04.94	128.53±00.72	190.07±1.00

Values are mean ± SEM; n= 06 in each group p was considered significant 95% CI

Table 2 depicting comparison between groups with p value* = <0.05

			ALP	SGPT	SGOT
	Group	**Group**	**P**	**p**	**P**
Tukey HSD	Control (I)	paracetemol	0.000*	0.000*	0.000*
		silymarine and paracetemol	0.130*	0.001*	0.011
		Acacia extract and paracetemol	0.062	0.001*	0.010*
	Paracetemol (II)	control	0.000	0.000	0.000
		silymarine and paracetemol	0.026*	0.000	0.002*
		Acacia extract and paracetemol	0.058	0.000	0.003*
	silymarine and paracetemol (III)	control	0.130	0.001*	0.011*
		paracetemol	0.026*	0.000	0.002*
		Acacia extract and paracetemol	0.979	1.000	1.000
	Acacia extract and paracetemol (IV)	control	0.062	0.001*	0.01*
		paracetemol	0.058	0.000	0.003*
		silymarine and paracetemol	0.979	1.000	1.000





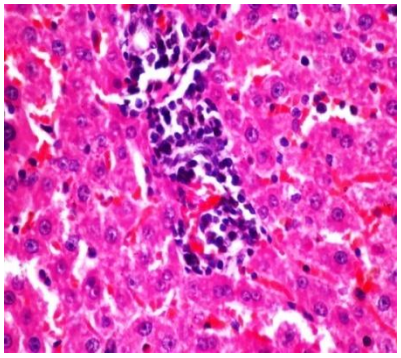


Fig 1, 2 and 3: light microscopic view of rat liver tissue after treatment with paracetemol

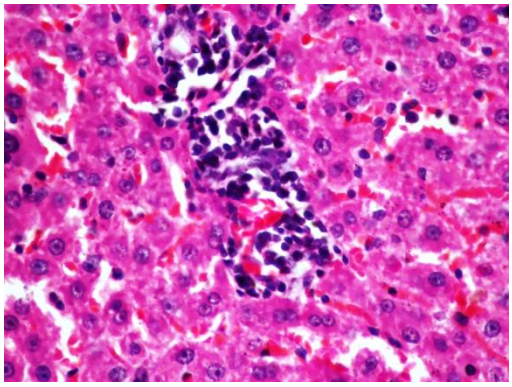
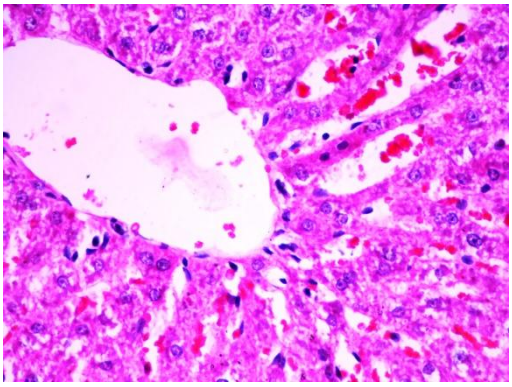



Fig 4and5: light microscopic view of rat liver tissue after treatment with Silymarin

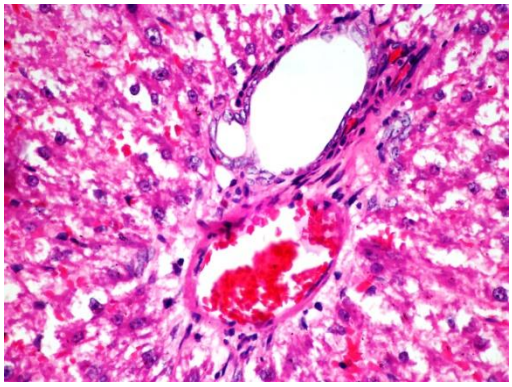
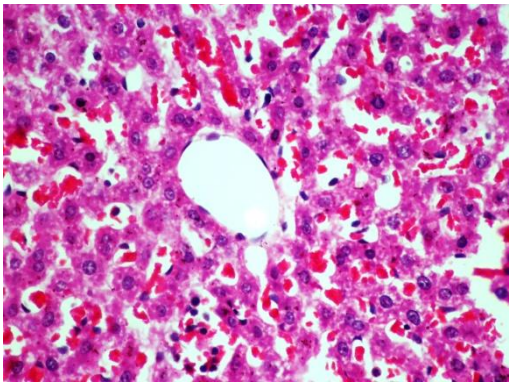



Fig 6 and 7: light microscopic view of rat liver tissue after treatment with acacia catechu extract

In **Figure 1, 2 & 3** shows histological morphology of rat liver which are treated with paracetemol. There is congestion, centrilobular hepatocyte necrosis and mild ballooning degeneration. In **Figure 3**, portal tract shows lymphocytic and plasmacytic infiltration, ballooning degeneration of hepatocyte along with generalised congestion. **Figure 4 & 5** group III rats which were treated with Silymarin along with paracetemol show mild congestion of central vein and mild ballooning degeneration of hepatocyte with normal looking portal triad. **Figure 6 & 7** group IV rats which were treated with acacia catechu extract show central vein with normal looking hepatocyte and portal tract with mild congestion.

DISCUSSION

Paracetemol, one of the most commonly used analgesic and antipyretic drug. Liver injury can be induced in experimental animals using paracetemol, carbontetrachloride, ethanol or any other hepatotoxic agent. There are several animal models have been shown to be effectively induce hepatotoxicity in animals [5].

In our study paracetemol was used to induce hepatotoxicity in experimental animal i.e. rats. After administration of paracetemol there was significant increase in serum enzyme SGOP, SGPT and ALP levels and characteristic histological changes as depicted in **Figures 1, 2 And3**, thus supporting the evidence of paracetemol induced hepatotoxicity.

Paracetemol is primarily metabolised in the liver by conjugation with glucoronic acid (about 60%) and, sulphuric acid (35%) or cysteine (~3%). Small amounts of hydroxylated and deacetylated metabolites also have been detected. Paracetemol also undergoes cytochrome (CYP) enzyme medicated N-hydroxylation to form highly reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI). Normally, harmful actions of this metabolite is nullified by reaction with sulfhydral groups present in glutathionine, but in higher doses of paracetemol there is excess production of NAPQI leading glutathione depletion from hepatocyte. Depletion of glutathione levels renders hepatocytes highly susceptible to oxidative stress and apoptosis. Hence there is NAPQI mediated lipid peroxidation leading to dysfunction of enzymatic

systems, structural cellular changes leading to hepatic damage [6, 7].

Histological examination of liver sections from group –II (paracetemol group) show congestion, cnrilobular hepatocyte necrosis and mild ballooning degeneration as depicted in figures 1,2 and3 confirming the hepatotoxic effects of paracetemol. These changes were positively co-related with the increase in serum enzyme levels ($p<0.05$) compared to control group-I. SGPT & SGOT and ALP are more commonly used biomarkers to study the hepatic injury. SGOT and SGPT are sensitive indicators of hepatocyte function, hence marked increase indicative of hepatic tissue injury by paracetemol in group –II [8, 9]. Silymarin a proven hepatoprotective was used as positive control [10].Silymarin a flavonoid known to be a hepatoprotective but exact mechanism of action is not known. It has been proposed to be an antioxidant, regulator of intracellular glutathione levels and cell membrane stabilizer [11]. It was administered at the dose of 25mg/kg orally in the form of suspension, showed a significant decrease in the serum transaminase levels as compared to group-II paracetemol alone ($P<0.05$). Histopathological study of Silymarin group (group-III) shows mild congestion as depicted in figures 4 & 5. Thus, confirming its hepatoprotective action. Group-IV animals which were administered with the ethanolic extract of acacia catechu at the dose of 250mg/kg orally, showed a marked decrease in serum enzyme levels as compared to paracetemol alone (group-II) with a significant level of difference $p<0.05$. Thus showing hepatoprotective action which was also supported by Histopathological changes i.e. normal looking hepatocyte, portal tract with mild congestion as shown in figure 6 & 7. The laboratory values were comparable with that of the Silymarin group without any statistical significant difference. Thus, confirming the hepatoprotective action of acacia catechu.

Acacia catechu is a deciduous, thorny tree which grows up to 12-15 meter in height. In Hindi is called as Khair , Kathu in kannada, kattha in urdu, khadir in Sanskrit and in English called as Indian peniunsula cutch [12]. Traditionally it is been used in healing wounds, as antimicrobial, anti-inflammatory and antifungal [13]. Also has been used in treatment of bleeding, treatment of diarrhoea and as astringent. Concentrated extracts mainly contain tannins (2-20%), catechins(25-33%), phlobotannins like catechutannic acid 20-50%, flavonoids, gums, resins and pigments.

Tannins have astringent action, whereas catechins have hepatoprotective, anti-oxidant, antdiarhoeal and hypoglycaemic action [2]. In our study phytochemical analysis showed presence of tannins, alkaloids, proteins and resins. From the study results, which shows a significant hepatoprotective activity, which was comparable with that of the standard control Silymarin. This significant action is supported by serum biochemical markers and histopathological examination. Thus the protective action can be attributed towards the presence of tannins, catechins and flavonoids which are known to have anti-oxidant and free radical scavenging action leading to hepatoprotection [14, 15, 16].

CONCLUSION

Thus it can be concluded that the ethanol extract of acacia catechu possesses a significant hepatoprotective effect. Limitation of our study was we could not isolate active molecule and its structural analysis. Hence studies to establish the exact mechanism of hepatoprotective action at cellular level can be undertaken.

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