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HEPATOPROTECTIVE ACTIVITY OF *AVERRHOA CARAMBOLA, CAJANUS CAJAN AND PAEDERIA FOETIDA* AGAINST ACETAMINOPHEN AND D-GALACTOSAMINE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT
Ripe fruits of *Averrhoa carambola* Linn., juice of tender leaves of *Cajanus cajan* Linn. and *Paederia foetida* Linn. are used by different ethnic communities of Assam for the treatment of jaundice, dyspepsia and other hepatic diseases. The hepatoprotective activity of the three plants species were studied against acetaminophen and D-galactosamine induced hepatotoxicity in experimental rats. Methanolic extracts (100mg/kg, p.o.) of ripe fruits of *Avorrhoa carambola* and tender leaves of *Cajanus cajans* attributed reduction of elevated biochemical enzymes viz. serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum total bilirubin (STBr). The experimental animal pre exposed to the extracts of *A carambola* and *C cajan* exhibited 32.07 - 35.38 and 68.28 - 75.86 of overall percentage reduction (OPR) of elevated biochemical enzymes. The post treatment with plant extracts exhibits OPR of 39.37 - 48.54 and 64.92 - 72.02 by *A carambola* and *C cajan* respectively. The comparative studies of histopathology of liver of the experimental rats corroborate to the biochemical finding. However, the extracts of *P foetida* failed to reverse the elevated liver specific biochemical enzymes as well as histopathological damage induced by both hepatotoxins in experimental rats. These evidences conclusively support that the *A carrambola* and *C cajan* possess moderate to good hepatoprotective activity.

Keywords: *Averrhoa carambola; Cajanus cajan; Paederia foetida; hepatoprotective activity; acetaminophen; D-galactosamine.*

INTRODUCTION

Assam is considered as floristic gateway of northeastern India for its rich diversity of flora and fauna [1,2]. The State is inhabited by more then 27 major ethnic communities[3]. These ethnic communities follow their own customs, traditions and folklore based health care system. The ethno pharmacological survey report shows that the herbal based folklore remedies are extensively practiced by different ethnic communities of Assam for the treatment of various diseases associated with liver. Literatures indicates that more than 49 species of plants are commonly used either alone or in combination of more than one plant in the treatment of common diseases associated with liver viz. jaundice, dyspepsia, inflammation and enlargement of liver[4-6].

Ripe fruit of *Averrhoa carambola* Linn. (Rutaceae; *Assamese - Kordoi*) is used in the treatment of infective jaundice including dyspepsia[1,4]. The ripe fruit is considered as a good remedy for bleeding piles and possess anti-scorbutic, cooling and refrigerant property. It is also used in dysentery, hepatic colic and diarrhea. The leaves of the plant is considered as antipyretic and also used in scabies. The juice obtained from the root is administered as antidote in case of poisoning[7].

The fresh juice of tender leaves of *Cajanus cajan* (L.) Mill. (Leguminace; *Assamese - Rahor mah*) is used in the treatment of acute infective jaundice [4,7,8]. The P*ederia foetida* Linn. (Rubiaceae; *Assamese - Vedai lata / Paduli lota*) a slender wiry and woody climber is extensively used in folklore remedies in the treatment of various liver diseases like infective hepatitis, inflammation of liver and jaundice. The decoction prepared from leaves is considered as wholesome and nutritive particularly for recovery after childbirth and for lactating mothers. The plant is also considered as a tonic, diuretic and good for liver and stomach disorders. The juice of the root is also prescribed to relieve pain in liver and inflammation of spleen [4,7].

In vivo investigation has been undertaken to evaluate the antihepatotoxic potency of methanolic extracts of ripe fruits of *A carambola*, tender leaves of *C cajan* and *P foetida* against acetaminophen and D-galactosamine induced hepatic lesion in experimental rats.

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Hepatoprotective Activity

MATERIALS AND METHODS

Plant material

The ripe fruit of *Averrhoa carambola* are collected locally in the month of August. The fresh ripe fruits are cut into small pieces and dried under sunlight. The fresh tender leaves of *Cajanus cajan* and *Paederia foetida* are collected in the month of April from home grown orchard and Jokai reserved forest of Dibrugarh District. The leaves are allowed to dry in shade.

All plant species were identified and authenticated with the help of relevant literature and reference herbarium specimens of the Institute of Pharmacy, Assam Medical College, Dibrugarh, Assam.

Experimental

Preparation of plant extracts

The dried plant materials were made into coarse powder in mechanical cutting machine and passed through a sieve (# 44) to obtain a uniform size. The powdered plant materials were subjected to cold methanolic extraction by macerating of 200g of powder in 1 liter of methanol for 3 successive times for 36 hours duration each. The solvents were removed from the extracts in rotary vacuum evaporator and the extracts were dried under reduced pressure in vacuum desiccator.

Reagent and chemicals

Acetaminophen and D-galactosamine for induction of hepatic injury were procured from M/s Fluka and M/s Sigma Aldrich (India) respectively. The solvents used for extraction of plant material were of GR grade of BDH and procured from local authorized agent. Silymarin (M/s Ranbaxy Laboratories Ltd, India) were used as reference drugs for calibration of hepatoprotective activity of tested plant extracts.

Experimental animals

Non-fasted Wister albino rats of both sex (200 - 220 g) were obtained from the central animal house of Assam Medical College, Dibrugarh, Assam. Prior to experimentation, the rats were separately housed and maintained at ambient room temperature for 90 days with standard diet and water *ad libitum.*

The experimental rats were divided into 6 groups viz., control (A), toxin (B) (acetaminophen / D-galactosamine), toxin with plant extract(C-E) and toxin with silymarin(F) comprising of 7 rats in each group.

Induction of hepatic lesions

Pre-treatment with plant extract in acetaminophen and D-galactosamine induce hepatic injury

The extracts of the three plant species were suspended in normal saline and administered (100mg/Kg) per orally 48 hours, 24 hours and 2 hours before administration of acetaminophen (3g/Kg,p.o.) and D-galactosamine (800mg/kg,i.p.) separate toxin treated groups of animals in two model experiments[9,10]. The control group of animals received normal saline and standard group of animals received silymarin (100mg/Kg,p.o.) in normal saline instead of plant extracts . The animals were sacrificed 24 hours after administration of hepatotoxin for evaluation of serum enzymes.

Post-treatment with extracts in acetaminophen and D-galactosamine induce hepatic toxicity

The test group of animals were administered 3 consecutive doses of plant extract of the three plant species at 1hr, 12 hr and 24 hr after administration of single dose of acetaminophen (3g/kg,p.o.) or D-galactosamine (800mg/kg,i.p.)[11,12]. The control group of animals received normal saline and animals of group-F of animals received silymarin (100mg/Kg, p.o.) instead of plant extract. The animals were sacrificed 24 hours after administration of last dose of plant extract for assay of serum enzymes.

Assessment of liver functions

The rats of experimental groups were anaesthetized with urethane (1.2g/kg, i.p.) of 25%w/v aqueous solution for collection of blood from carotid artery. The collected blood was allowed to coagulate at 37ºC for 30 minutes. The serums of the blood were separated by centrifugation at 2500 rpm and immediately processed for estimation of SGOT, SGPT and STBr.

The analytical reagents used for estimation of serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total serum bilirubin (STBr) were carried out using *in vitro* kits (Ecoline) made by M/s Marck, India Ltd. Analysis of SGOT,SGPT and STBr were performed in biochemical analyzer (Micro lab-300, M/s Merck, India Ltd).

Statistical analysis

The results of all the biochemical parameters are expressed as mean ±Standard Deviation (±SD). In order to check the pairs of means which were different, the data for every biochemical parameter in each set was analyzed by least significant difference (LSD) procedure at 95%
(P = 0.05) confidence level [13,14]. The value of estimated serum biochemical parameters, viz. SGOT, SGPT and STBr were represented by overall percentage reduction (OPR) with respect to control group as 100% reduction.

RESULTS

The changes of marked serum enzymes and representative photographs of histo-pathological study of liver in experimental rats on administration of methanolic extracts of the three plant species on pre and post treatment schedules in acetaminophen and D-galactosamine induced liver injury are placed in Table 1 – 4 and Fig 1 & 2.

Hepatoprotective Activity

Table 1 *: Effect of plant extracts on rats intoxicated with acetaminophen on serum enzyme - Pre-treatment with plant extracts*

Groups	SGPT(µl)	SGOT(µl)	STBr(mg/dl)	OPR
A.Control	56.68 ± 7.43	91.72 ± 6.54	0.22 ± 0.08	100
B.Acetaminophen	200.75 ± 10.17**	240.25 ± 6.52**	2.12 ± 0.21**	-
C.*A.carambola*	154.68 ± 9.32**	182.90 ± 7.83**	1.33 ± 0.21**	35.38
D.*C.cajan*	106.12 ± 5.99**	135.17 ± 7.10**	0.72 ± 0.17**	68.28
E.*P.foetida*	189.08 ± 8.51*	227.5 ± 10.49*	1.87 ± 0.21	8.17
F. Silymarin	96.65 ± 7.67**	116.45 ± 6.23**	0.53 ± 0.08**	77.92
Significant difference between various groups, p=0.05	A & B – F B & C – F C & D – F D & E E & F	A & B – F B & C – F C & D – F D & E,F E & F	A & B – F B & C – F C & D – F D & E E & F	

Table 2 *: Effect of plant extracts on rats intoxicated with acetaminophen on serum enzyme - Post -treatment with plant extracts*

Groups	SGPT(µl)	SGOT(µl)	STBr(mg/dl)	OPR
A.Control	56.68 ± 7.43	91.72 ± 6.54	0.22 ± 0.08	100
B.Acetaminophen	206.70 ± 5.94**	242.87 ± 6.45**	2.20 ± 0.19**	-
C.*A.carambola*	139.78 ± 7.99**	163.77 ± 7.83**	1.05 ± 0.19*	48.54
D.*C.cajan*	118.65 ± 4.80**	126.65 ± 2.72**	0.58 ± 0.17**	64.92
E.*P.foetida*	169.10 ± 6.93*	191.50 ± 7.36	1.78 ± 0.19	29.48
F. Silymarin	103.77 ± 7.83**	115.37 ± 5.67**	0.45 ± 0.10**	96.88
Significant difference between various groups, p=0.05	A & B – F B & C – F C & D – F D & E,F E & F	A & B – F B & C – F C & D – F D & E,F E & F	A & B – F B & C – F C & D – F D & E E & F	

**P< 0.05, ** P< 0.01; n = 6, Dose: Acetaminophen 3g/kg,p.o. Plant extracts and silymarin 100 mg/kg,p.o.*

Table 3 *: Effect of plant extracts on rats intoxicated with D - galactosamine - Pre-treatment with plant extracts*

Groups	SGPT(µl)	SGOT(µl)	STBr(mg/dl)	OPR
A.Control	56.68 ± 7.43	91.72 ± 6.54	0.22 ± 0.08	100
B.Galactosamine	194.00 ± 8.52**	233.67 ± 7.38**	1.82 ± 0.15**	-
C.*A.carambola*	162.77 ± 7.07**	175.63 ± 7.55**	1.00 ± 0.31**	32.07
D.*C.cajan*	92.93 ± 7.80**	122.82 ± 8.80**	0.65 ± 0.10**	75.86
E.*P.foetida*	183.00 ± 7.18	227.32 ± 8.22	1.65 ± 0.24	6.23
F. Silymarin	83.82 ± 8.18**	109.48 ± 7.00**	0.53 ± 0.08**	83.90
Significant difference between various groups, p=0.05	A & B – F B & C – F C & D – F D & E,F E & F	A & B – F B & C,D,F C & D – F D & E,F E & F	A & B – F B & C,D,F C & D – F D & E E & F	

Table 4 : *Effect of plant extracts on rats intoxicated with D - galactosamine - Post-treatment with plant extracts*

Groups	SGPT(µl)	SGOT(µl)	STBr(mg/dl)	OPR
A. Control	56.68 ± 7.43	91.72 ± 6.54	0.22 ± 0.08	100
B. Galactosamine	201.87 ± 7.89**	236.82 ± 8.03**	1.90 ± 0.09**	
C. *A. carambola*	140.32 ± 8.06**	184.00 ± 9.69**	1.30 ± 0.11**	39.37
D. *C. cajan*	92.68 ± 9.96**	121.45 ± 5.92**	0.82 ± 0.12**	77.02
E. *P. foetida*	184.68 ± 10.15	218.05 ± 10.62	1.70 ± 0.14*	12.38
F. Silymarin	85.18 ± 8.12**	108.67 ± 5.57**	0.65 ± 0.10**	84.28
Significant difference between various groups, p= 0.05	A & B – F B & C – F C & D – F D & E E & F	A & B – F B & C – F C & D – F D & E,F E & F	A & B – F B & C – F C & D – F D & E,F E & F	

**P< 0.05, ** P< 0.01 ; n = 6,*
Dose: D - galactosamine 800mg/kg,p.o.
Plant extracts and silymarin 100 mg/kg,p.o.

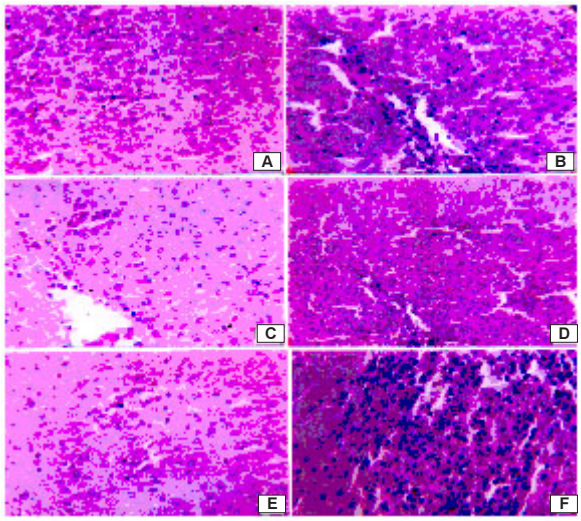

Fig. 1 *: Representative photographs of histopathological changes on the liver of rats on treatment of plant extracts intoxicated with D- galactosamine.*
(A - Control, B- D galactosamine,C- A carambola, D- C cajan, E-silimarin , F- P foetida)

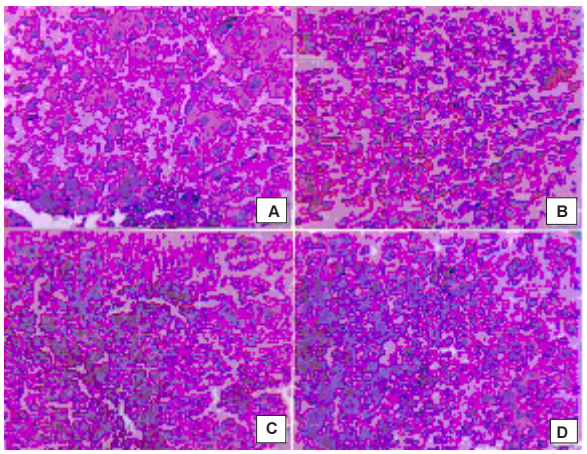

Fig. 2 *: Representative photographs of histopathological changes on the liver of rats on treatment of plant extracts intoxicated with acetoaminophen.*
(A - Acetaminophen, B – A. carambola, C – C. cajan, D - P. foetida)

DISCUSSION

The result in Table 1-4 shows that there are significant increase of serum enzymes viz. SGOT, SGPT and STBr in toxin treated animals due to hepatic lesion produced by acetaminophen and D-galactosamine. The plant extracts have attributed varied intensity of hepatoprotective activity in the pre and post treatment schedule against two hepatotoxins as evident from the reversal of elevated serum enzyme.

In pre-treatment schedule, the extracts of *C cajan* and *A carambola* exhibited 77.92 to 35.38 OPR Of elevated enzyme in encountering the liver toxicity induced by acetaminophen and D-galactosamine. But, extract of *P foetida* has failed to encounter hepatic lesion

produced by two hepatotoxins as evident from the insignificant reduction of elevated serum enzymes.

In the post treatment schedule, the extracts of *C cajan* has shown to be the most efficacious indicating 64.92 to 77.02 OPR among the three extracts followed by the extract ripe fruits of *A carambola.* In post- treatment protocol too, the extract of *P foetida* has failed to rescission significantly the elevated liver specific enzymes.

Although, the serum level of SGOT, SGPT and STBr were not normalized completely in experimental rats, but these three biochemical parameter were lowered significantly by the methanol extracts of *A carambola* and *C cajan* in both models. The concoction of the results in Table 1 - 4 demonstrates that there are concordances of hepatoprotective efficacy of extracts of three plant spices in D-galactosamine and acetaminophen induced hepatic toxicity in two experimental models.

The moderate to good hepatoprotective effect on treatment with 100mg of extract of *A carambola* and *C cajan* were further confirmed by histopathological examination of the liver of the control, D-galactosamine and acetaminophen treated and plant extract treated group of rats (Fig: 1 & 2).The liver of D-galactosamine treated rats (Fig:1B) showed focal necrosis especially in the periportal area predominantly micro vesicular fatty changes were observed and there was infiltration by lymphocytes and neutrophils in galactosamine treated rats. In contrast, the extract of *A Carambola, C cajan* and silymarin treated rats (Fig:1C&D) retained normal hepatic architectural pattern quite close to the liver of the control group of rats.

In case of acetaminophen induced lesion in liver of rats there are gross necrosis of the centribular hepatocytes characterized by nuclear pyknosis, karyolysis and eosinophilic infiltration (Fig. 2A). Administration of plant extracts of *A carambola and C cajan* reversed to a large extent the hepatic lesion as is obvious from the absence of eosinophilia and presence of fewer necrosis zone and kupffer cells in the liver of rats treated with extracts of *A carambola* and *C cajan* (Fig.2B&C). However extracts of *P foetida* failed to reverse the histological damage caused by the two hepatotoxins (Fig-1 F & Fig-2 D)

In the present study, D-galactosamine and acetaminophen was employed for induction of hepatic necrosis in experimental animals. These two hepatotoxins produce wide range of hepatic injuries by different mechanism thereby significantly elevating the liver enzymes in serum. The mechanism involves in induction of hepatic damage by acetaminophen and galactosamine is significantly unobtrusive as far as pathophysiology is concerned.

The acetaminophen induced hepatotoxcity characterized by marked elevation of liver specific enzymes in serum due to acute liver tissue damage through cholestatic effects[15] which is attributed during its metabolism to the formation of toxic metabolites activated through cytochrome P450 to highly reactive metabolites N-acetyl- p-benzoquinoneimine (NAPQI). NAPQI binds to macromolecules of hepatic cell and this leads to the death of cells[16,17]. However, galactosamine is very specific in producing steatosis liver type injuries. Metabolism of D galactosamine in the liver causes lowering of the level of uracil nucleotide resulting inhibition of RNA synthesis in hepatocytes [18,19].

In pre and pos-treatment models, the extracts of *C cajan* and *A carambola* demonstrates reasonably good to moderate prophylactic and therapeutic potency against two hepatotoxins. In both pre and post-treatment models, the extracts of *C cajan* and *A carambola* afforded similar potency and their efficacy is near normal of equal dose of silymarin. These observations validate the folklore claims of the two plant species in the treatment of various diseases associated with liver.

In most of the folklore based herbal remedies used in the treatment of liver diseases the crude extract, juice and decoction of more than one plant species are mixed up in different ratio by traditional healer. Such combination may be considered as crucial to obtain desired benefit and optimum therapeutic efficacy of folklore and herbal medicines. Although, the extract of *P foetida* failed to encounter inflated enzymes, the plant may have beneficial role in curing liver ailments when used in combination with other herbal remedies.

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