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Hepatoprotective activity of *Averrhoa carambola* against paracetamol induced liver toxicity in mice

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Abstract
Liver is considered to be the master gland of the body as it is involved in various biological functions such as growth, immunity, energy supply, detoxification, reproduction etc. Therefore, maintenance of a healthy liver for normal functioning of human body is highly essential. Liver cells are damaged by various exogenous agents like microorganisms, chemotherapeutic agents and alcohol. The Indian traditional medical system suggests many plants for the treatment of liver ailments. A plenty of plant families are still unexplored for their bioactive potentials. The objective of the present study was to evaluate the hepatoprotective effect of methanolic extract of *Averrhoa carambola* on paracetamol-induced liver damaged Swiss albino mice. Administration of plant extract (200 mg/kg and 400 mg/kg) significantly reduced the effect of paracetamol-induced toxicity on the blood serum markers of liver damage such as Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP) and Bilirubin. This study, therefore, showed the potential of *Averrhoa carambola* extract to reduce the levels of serum marker enzymes which indicate the protection of liver cells against paracetamol induced liver damage.

Keywords: Hepatoprotective, *Averrhoa carambola*, liver injury, paracetamol, liver enzymes

1. Introduction
Liver is an important organ which plays an essential role in the metabolism of biological macromolecules. Hence it is called as master gland of human body. Liver is affected by various kinds of diseases that cause numerous deaths worldwide. Global liver cirrhosis deaths increased from around 676,000 in 1980 to over one million in 2010 [2, 11]. Liver damage occurs due to infectious organisms, alcohol consumption and intake of certain drugs at higher doses [1]. Although, there are developments in the treatment of hepatic diseases, very few drugs are available which enhance liver function, provide protection to liver from damage or facilitate the regeneration of liver cells. Nowadays, plant-based drugs have gained momentum as they are cost-effective and eco-friendly in nature. The plant kingdom possesses rich sources of bioactive compounds that can be utilized for the treatment of numerous kinds of diseases. The Indian traditional medicine systems like ayurveda, unani, siddha and homeopathy are mostly dependent on the plant-derived formulations. The Western Ghats are endowed with an array of endemic plant species with medicinal importance. These resources have been traditionally exploited by the tribal people who live in these regions for several centuries [16]. In the present study, *Averrhoa carambola* plants were collected from Southern Western Ghats, Virudhunagar district, India. The methanolic extract of fruit of *Averrhoa carambola* was used to evaluate the hepatoprotective effect against paracetamol-induced liver toxicity in Swiss albino mice. The biochemical parameters such as Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP) and Bilirubin were determined. The results of the study were compared with sylimarin, which is a standard drug, and analyzed for significance by one-way analysis of variance (ANOVA).

2. Materials and Methods
2.1 Plant materials
The fruits of *Averrhoa carambola* (Oxalidaceae) were collected from Thiruolakkamparai, Western Ghats, Virudhunagar district, India. *Averrhoa carambola* is a slow-growing and short-trunked with a much-branched tree. The fruits are showy, oblong, longitudinally 5-6 angled fruits with orange-yellow skin and juicy, crisp, yellow flesh when fully ripe. They were identified by Dr. R. Ramasubbu, (a plant taxonomist) of the Department of Biology, Gandhigram Rural Institute, Dindigul, India. The voucher specimens were deposited in the Department of Botany Herbarium, VHNSN College, Virudhunagar, India for future references. The fruits were washed with water and air dried in the shade until the complete evaporation of water. The dried fruits were pulverized with a mixer grinder into fine powder. 200 g of powdered fruits were successively extracted with chloroform, methanol and water in a Soxhlet apparatus for 72 hours. The methanolic extract alone was selected for pharmacological screening. It was filtered through muslin cloth and concentrated at 40°C in vacuum.

2.2 Chemicals
The organic solvents were purchased from Merck (India), Mumbai. Paracetamol (Cipla Ltd., Mumbai) and Sylimarin (Lupin Laboratories Ltd., Mumbai) were procured from local market. All other chemicals used in the present study were of analytical grade.

2.3 Animals
Twenty male Swiss albino mice, weighing 110-150 g, were used in the pharmacological studies. The animals were housed in polypropylene cages which were kept in a well ventilated room with natural 12 hr light/ dark cycle. During the experimental period, they were fed with standard diet

obtained from Poultry Research Station, Chennai and provided with water *ad libitum*. They were allowed to acclimatize the laboratory conditions for two weeks. The experimental protocol was approved by the animal ethics committee constituted for the purpose as per CPCSEA guidelines.

2.4 Experimental design for hepatoprotective activity

The animals were divided into 5 groups each having 4 mice. Group I animals received distilled water for seven days which served as normal control. The group II animals were treated with Paracetamol (2g /kg body weight) dissolved in glucose water and served as negative control. The animals of Group III and IV were administered with Paracetamol (2g /kg body weight) and *Averrhoa carambola* extract at 200 mg/kg and 400 mg/kg body weight respectively for seven days orally [18]. Group V animals were orally administered with the standard drug Silymarin (20 mg/kg body weight) and Paracetamol (2g /kg body weight) for seven days and served as positive control. On the eighth day, the animals were sacrificed by mild ether euthanasia. Blood samples from each group were collected by cardiac puncture separately in sterile centrifuge tubes and allowed to clot for 30 min at 37 °C. The coagulated blood samples were centrifuged at 3000 rpm (Remi, Mumbai) for 10 min. The clear serum thus obtained was used for the estimation of Serum Glutamic Pyruvic Transaminase (SGPT) [17], Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP) and Bilirubin [10].

The results are presented as Mean ± Standard Error of Mean (SEM) of four animals in a group. The data were analyzed by one way ANOVA using SPSS 12.0. The P-value <0.05 was considered as statistically significant.

3. Results

The present study was aimed to evaluate the hepatoprotective effect of methanolic extract of *Averrhoa carambola* fruit in paracetamol-induced liver toxicity at different doses and the results are presented in Table. 1. It is evident that there was an increase in SGPT, SGOT, ALP and bilirubin levels in the paracetamol intoxicated animals compared to normal control. It is observed that the plant extract exhibited a significant protection against paracetamol-induced liver injury in mice as expressed by the reduction in toxin-mediated rise of serum markers. The groups which received *Averrhoa carambola* fruit extract showed significant decrease in the level of liver enzymes. But the changes were not identical in both the groups. The groups that were administered with 200 mg (kg body weight) of plant extract showed lower response than the animals treated with 400 mg (kg body weight) of *Averrhoa carambola* fruit extract. The serum level of SGOT in the paracetamol administered group increased significantly (83%) than normal control group. In this group, the bilirubin increased to 2.84 mg/dl which is 139% higher than the control. The plant extract of *Averrhoa carambola* at a dose of 400 mg/ kg body weight exhibited highly significant changes. The level of various biochemical markers are very close to the Sylimarin treated groups. A lowest percent of recovery was observed in the animals treated with the plant extract at the dose of 200 mg/ kg body weight. But it showed the elevated level of marker enzymes than Group IV and Group V.

4. Discussion

The induction of liver damage by paracetamol in this study was adopted because it is well known that paracetamol causes severe hepatic injury as evidenced by several earlier reports [3, 6, 14, 22]. The paracetamol administration significantly increased the serum enzyme levels viz., SGOT, SGPT, ALP and Bilirubin. These biomolecules are considered to be ideal markers for the diagnosis of various kinds of liver disorders. When the plasmamembrane of liver cell is damaged by paracetamol, the enzymes present in the cytoplasm are released into the blood. This phenomenon can be exploited to assess the type and extent of liver damage [18]. Increased level of liver enzymes in the serum of paracetamol treated animals is attributed to liver damage as these enzymes leak out of hepatocyte and causes hepatonecrosis [5, 13, 21]. In addition to several other functions, the liver excretes bilirubin as break down product of hemoglobin through bile secretion. The injury caused by paracetamol in liver also affects the parenchyma cells which results in elevated level of bilirubin in the serum [19]. On the other hand, the *Averrhoa carambola* extract treated groups showed a declining trend in the level of enzyme markers. As evident from Table 1, the biochemical parameters of control group is significantly higher than the extract-treated groups. It shows that the plant extract had influenced the functions of injured liver. In an earlier study, it was found that the structural integrity of the liver cell membrane was preserved in rats which were subjected to paracetamol-induced hepatotoxicity followed by the treatment with hydroalcoholic extract of *Alocasia indica* (Linn.) [12]. It was also observed that the effect was in a dose dependent manner. In the histological examination of liver sections of rats treated with plant extracts, the normal cellular architecture was retained. Similar trend was observed in the animals treated with the standard drug Sylimarin [4]. These results conform to the present study also as the *Averrhoa carambola* extract treated groups showed hepatoprotective effect. Pre-treatment with plant extract restored the liver enzyme parameters thereby conferring protection to liver against paracetamol-induced toxicity. The decrease in serum levels of these enzymes may be attributed to the presence of bioactive components like phenolics and flavonoids in the fruit extract of *Averrhoa carambola* that caused the regeneration of liver tissue. It is well established that the flavonoids possess free radical scavenging properties [7, 15]. The hepatoprotective effect of naringenin, a flavonoid, was investigated in rats against ethanol-induced liver injury. Supplementation with naringenin to ethanol-fed rats significantly decreased the levels of bilirubin, ALP, lactate dehydrogenase (LDH), conjugated dienes (CD) and phase I enzymes [8, 9]. It was observed that the level of superoxide dismutase (SOD), catalase (CAT), alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) increased significantly in rats with ethanol treated hepatic damage when compared with untreated animals.

5. Tables

Table 1: Effect of methanolic extract of *Averrhoa carambola* fruit on various biochemical parameters in Swiss albino mice with paracetamol-induced hepatotoxicity

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Bilirubin (mg/ dl)
Control (distilled water)	75.07±0.44	82.24±0.95	14.79±0.21	1.19±0.02
Paracetamol (2 g/ kg)	134.61±2.19	150.37±0.78	87.42±0.67	2.84±0.08
Paracetamol + AC Extract (200 mg/ kg)	106.10±2.36*	120.01±0.41*	39.93±0.62*	1.85±0.07*
Paracetamol + AC Extract (400 mg/ kg)	79.00±0.63**	84.43±1.27**	21.26±0.44**	1.25±0.03**
Paracetamol + Sylimarin (20 mg/ kg)	77.42±0.66**	82.90±1.33**	16.76±0.17**	1.23±0.01**

The values are expressed as mean ± SEM (n = 4 mice/ group). The symbols * and ** represent highly significant levels at $p<0.05$ and $p<0.01$ respectively when compared with negative control

6. Conclusion

Hence, it can be concluded that methanolic extract of *Averrhoa carambola* fruit possess hepatoprotective effect against paracetamol-induced liver damage in Swiss albino mice as evidenced by serum enzyme markers. The hepatoprotective potential may be due to the presence of bioactive principles such as phenolics, flavonoids and other phytochemical constituents present in the extract. In this line, further work is needed to identify and isolate the constituents for the determination of actual protective mechanism.

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8. References

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