

## Effect of *Psidium guajava* Linn. leaf extract on liver cells

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**ABSTRACT** The study was designed to evaluate the hepatoprotective activity of *P. guajava* in acute experimental liver injury induced by carbon tetrachloride, paracetamol or thioacetamide. The effects observed were compared with a known hepatoprotective agent, silymarin. In the acute liver damage induced by different hepatotoxins, *P. guajava* leaf extracts (250 and 500mg/kg, p.o) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. The higher dose of the extract (500 mg/kg, p.o) prevented the increase in liver weight when compared to hepatotoxin treated control, while the lower dose was ineffective except in the paracetamol induced liver damage. Histological examination of the liver tissues supported the hepatoprotection. It is concluded that the aqueous extract of leaves of guava plant possesses good hepatoprotective activity.

**Keywords:** Carbon tetrachloride, Hepatoprotection, Paracetamol, *Psidium guajava* Linn., Thioacetamide

### Introduction

Liver diseases, such as jaundice, cirrhosis and fatty liver are very common worldwide. In India, numerous medicinal plants are used for the treatment of liver disorders. One of the plants used traditionally is guava plant, *Psidium guajava* Linn (Myrtaceae). It is believed in folklore that the water decoction of the leaves of this plant can cure jaundice within three days. It is used widely in Mangalore district of Karnataka. *Psidium guajava* contains a number of chemical constituents, which are reported to possess antibacterial<sup>1</sup>, anti-diarrheal<sup>2</sup>, antimycobacterial<sup>3</sup>, antihyperglycemic<sup>4</sup>, antimalarial<sup>5</sup>, cytotoxic<sup>6</sup> and antioxidant activities<sup>7</sup>. The anti-oxidant activity is due to presence of a number of constituents; the major ones are caryophyllene oxide, caryophyllene and a number of tannins<sup>8</sup>. Since, anti-oxidants are known to reduce the development of chemically induced liver damage; the effect of water decoction of the leaves of the plant *P. guajava* has been evaluated for hepatoprotective activity.

### Experimental

#### Animal

Wistar albino rats weighing 175-250 g of either sex were used. The experimental protocol was approved by the Institutional Animal Ethics Committee and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

#### Extraction of *Psidium guajava* leaves

The fresh leaves of *P. guajava* were collected during November from Koramangala area in Bangalore. The Regional Research Institute, Bangalore identified and authenticated the plant. A specimen (RRCBI, Acc. No. 12473) has been preserved for future reference. The fresh leaves were ground using mortar and pestle and distilled water. For each 100 g of crushed leaves, 300 ml of water was added. The crushed leaves were soaked in water for about 24 hrs. After 24hrs the water was strained through a muslin cloth. The aqueous extract obtained was evaporated in rotary evaporator to get a powdery mass. The yield was 4.6% (w/w). The powder obtained was then subjected to phytochemical analysis to determine the chemical

constituents present in the extract. The powdery extract of *P. guajava* leaves (PGJ) was suspended in water without adding any suspending agent for oral administration.

#### **Acute toxicity study**

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticide and Toxic Substance) Up and Down procedure<sup>9</sup>.

*Evaluation of hepatoprotective activity* - Hepatoprotective activity was evaluated using acute hepatic injury models induced by carbon tetrachloride, paracetamol or thioacetamide.

#### **Acute hepatitis models**

##### **Carbon tetrachloride (CCl<sub>4</sub>) induced acute toxicity**

The CCl<sub>4</sub> was diluted with liquid paraffin (1:1) before administration. The animals were divided into 5 groups consisting of 6 animals for each. The animals were then subjected to either one of the following treatments for 9 days.

Group 1: distilled water (1 ml/kg, p.o)

Group 2: distilled water for 9 days + CCl<sub>4</sub> (1ml/kg, p.o) on ninth day

Group 3: silymarin (100mg/kg /day, p.o) for 9 days + CCl<sub>4</sub> (1ml/kg, p.o) on ninth day

Group 4: PGJ (250mg/kg/day, p.o) for 9 days + CCl<sub>4</sub> (1ml/kg, p.o) on ninth day

Group 5: PGJ (500mg/kg/day, p.o.) for 9 days + CCl<sub>4</sub> (1ml/kg, p.o) on ninth day

Food was withdrawn 12 h before carbon tetrachloride administration to

## **Results**

### **Phytochemical investigation**

The preliminary phytochemical investigation of the aqueous extracts of the *P. guajava* showed that it contains carbohydrates, tannins, flavanoids, saponins, steroids, proteins and amino acids.

### **Acute oral toxicity study**

*Psidium guajava* Linn. aqueous extract (PGJ) up to a dose of 2000 mg/kg, p.o did not produce any mortality. Hence 1/4<sup>th</sup> and 1/8<sup>th</sup> of this dose i.e. 500 mg/kg and 250 mg/kg, p.o were used.

enhance the acute liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 hr after the administration of CCl<sub>4</sub>. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to histopathological examination<sup>10</sup>.

### **Paracetamol (PCM) induced liver toxicity**

The same procedure as mentioned above was followed except that the liver was damaged using PCM (1g/kg, p.o) diluted with sucrose solution (40% w/v). PCM was administered in 3 divided doses on day 9 and animals were sacrificed 48 hr after administration of PCM<sup>11</sup>.

### **Thioacetamide induced liver toxicity**

The same procedure was followed. Damage was induced by using TAA (100mg/kg, s.c), which was prepared in distilled water (2% solution)<sup>12</sup>.

### **Statistical analysis**

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The values are expressed as mean  $\pm$ SEM and  $P \leq 0.05$  was considered significant.

### **Carbon tetrachloride induced acute toxicity**

The higher dose of PGJ (500mg/kg p.o) and silymarin (100 mg/kg p.o) produced a significant reduction in serum marker enzymes ( $P \leq 0.001$ ). Lower dose of PGJ (250 mg/kg, p.o) also produced a significant reduction in ALT, AST, ALP and serum bilirubin when compared to CCl<sub>4</sub> treated group, but it was less effective. Administration of CCl<sub>4</sub> produced a non-significant increase in liver weight. Silymarin and the low dose of PGJ (250mg/kg, p.o) did not affect the liver weight, when

compared to CCl<sub>4</sub> treated control, whereas higher dose of PGJ (500mg/kg, p.o) showed a significant reduction in the liver weight ( $P \leq 0.05$ ) when compared with CCl<sub>4</sub> treated group (T<sub>1</sub>). Histological examination of the liver tissue from CCl<sub>4</sub> treated animals revealed that CCl<sub>4</sub> had produced profound inflammation and congestion especially in the sinusoids. Hydropic degeneration and steatosis in the periportal region was also observed. Pretreatment of animals with silymarin, PGJ (250 mg/kg, p.o) and PGJ (500 mg/kg, p.o) reduced the inflammation, degenerative changes and steatosis (F<sub>1</sub>).

#### Paracetamol induced liver toxicity

After 48 hr of administration of PCM, the serum levels of ALT, AST, ALP and bilirubin were markedly increased. Pretreatment with PGJ (500 mg/kg, p.o) and silymarin

significantly reduced the levels of biochemical markers when compared to PCM treated group ( $P \leq 0.001$ ). Pretreated with PGJ (250 mg/kg, p.o) did not show significant effect when compared with the PCM control. Pretreatment with PGJ (250 mg/kg, p.o) and silymarin significantly reduced the increase in the liver weight seen after PCM intoxication (T<sub>2</sub>). PCM produced severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis. Silymarin reduced the pyknosis of hepatocytes when compared to PCM treated control. Animals treated with both lower and higher dose of PGJ showed mild hydropic degeneration and there was no pyknosis or congestion (F<sub>2</sub>).

**Table 1:** Effect of silymarin and *P. guajava* leaf extract on serum ALT, AST, ALP, bilirubin levels and liver wet weight in CCl<sub>4</sub> induced acute liver injury in rats<sup>#</sup>.

Treatment	Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver weight (g/100g bw)
Vehicle control		72.86 ± 6.19	186.36 ± 7.81	398.18 ± 6.73	0.26 ± 0.08	3.12 ± 0.09
CCl <sub>4</sub> control		384.00 ± 25.02 <sup>a</sup>	642.20 ± 31.01 <sup>a</sup>	749.60 ± 36.25 <sup>a</sup>	1.56 ± 0.22 <sup>a</sup>	3.78 ± 0.30 <sup>b</sup>
CCl <sub>4</sub> + Silymarin	100	57.22 ± 2.81 <sup>***</sup>	205.46 ± 6.12 <sup>***</sup>	408.02 ± 5.58 <sup>***</sup>	0.28 ± 0.03 <sup>***</sup>	3.10 ± 0.06 <sup>ns</sup>
CCl <sub>4</sub> + PGJ	250	60.10 ± 7.70 <sup>**</sup>	572.66 ± 43.16 <sup>ns</sup>	539.96 ± 79.45 <sup>***</sup>	0.26 ± 0.11 <sup>***</sup>	3.18 ± 0.07 <sup>ns</sup>
CCl <sub>4</sub> + PGJ	500	16.72 ± 4.09 <sup>**</sup>	151.74 ± 13.95 <sup>***</sup>	488.82 ± 10.01 <sup>***</sup>	0.26 ± 0.05 <sup>***</sup>	2.99 ± 0.12 <sup>ns</sup>

<sup>#</sup>values are mean ± SE from 6 animals in each group; P values: <sup>a</sup>  $p \leq 0.001$  vs. vehicle control, <sup>b</sup>  $p \leq 0.05$  vs. vehicle control, <sup>ns</sup>  $p > 0.05$ , <sup>\*</sup>  $p \leq 0.05$ , <sup>\*\*</sup>  $p \leq 0.01$ , <sup>\*\*\*</sup>  $p \leq 0.001$  vs. CCl<sub>4</sub> treated control

#### Thioacetamide induced liver necrosis

A significant difference in serum biochemical markers was observed between normal and TAA treated group ( $P \leq 0.001$ ). Pretreatment of animals with PGJ (250 and 500mg/kg, p.o) and silymarin significantly reduced the levels of AST, ALT and ALP ( $P \leq 0.001$ ). PGJ at both the dose levels (250 mg/kg and 500mg/kg, p.o) did not affect serum bilirubin levels. TAA induced acute toxicity increased the weight of liver significantly ( $P \leq 0.01$ ). The higher dose of PGJ (500mg/kg, p.o) and silymarin prevented the increase in liver weight that

was observed in TAA treated group, while the lower dose of PGJ (250mg/kg, p.o) did not produce any significant decrease in liver weight (T<sub>3</sub>). Histological examination showed perlobular hepatocyte necrosis, inflammation and congestion with cytoplasmic vacuolation in TAA treated control animals. In silymarin treated animals, mild inflammation and mild necrosis of hepatocytes with cytoplasmic vacuolation was noted. Animals treated with lower dose showed periportal necrosis and those treated with higher dose showed mild inflammation and necrosis (F<sub>3</sub>).

## Discussion

The aqueous extract of *Psidium guajava* leaves showed good hepatoprotective activity when administered at doses of 250 mg/kg and 500 mg/kg orally and the effect observed was dose dependent. The effect produced by the higher dose of aqueous extract of *P. guajava* leaves was similar to that produced by silymarin (100mg/kg, p.o), a well known hepatoprotective agent.

CCl<sub>4</sub> is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl<sub>4</sub> are largely due to generation of free

radicals<sup>14</sup>. Drugs having antioxidant activity are effective in treating CCl<sub>4</sub> induced hepatotoxicity. PGJ is known to have antioxidant activity. Different extracts of this plant including the water extract are reported to increase the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH)<sup>7</sup>. The CCl<sub>4</sub> induced a significant increase in liver weight, which is due to blocking of secretion of hepatic triglycerides into the plasma<sup>15</sup>. Silymarin and PGJ (250mg/kg, p.o) did not prevent the increase of liver weight, whereas PGJ (500mg/kg, p.o) prevented the increase of liver weight in rats.

**Table 2:** Effect of silymarin and *P. guajava* leaf extract on serum ALT, AST, ALP, bilirubin levels and liver wet weight in paracetamol (PCM) induced acute liver injury in rats<sup>#</sup>.

Treatment	Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver weight (g/100g bw)
Vehicle control		72.86 ± 6.19	186.36 ± 7.81	398.18 ± 6.73	0.26 ± 0.08	3.12 ± 0.09
CCl <sub>4</sub> control		384.00 ± 25.02 <sup>a</sup>	642.20 ± 31.01 <sup>a</sup>	749.60 ± 36.25 <sup>a</sup>	1.56 ± 0.22 <sup>a</sup>	4.40 ± 0.14 <sup>a</sup>
CCl <sub>4</sub> + Silymarin	100	75.36 ± 4.25 <sup>***</sup>	167.34 ± 5.51 <sup>***</sup>	322.12 ± 6.94 <sup>***</sup>	0.24 ± 0.05 <sup>***</sup>	0.24 ± 0.05 <sup>***</sup>
CCl <sub>4</sub> + PGJ	250	98.28 ± 2.49 <sup>***</sup>	170.50 ± 5.13 <sup>***</sup>	624.28 ± 88.77 <sup>ns</sup>	0.48 ± 0.02 <sup>***</sup>	3.18 ± 0.07 <sup>***</sup>
CCl <sub>4</sub> + PGJ	500	86.94 ± 18.94 <sup>***</sup>	178.92 ± 8.07 <sup>***</sup>	338.32 ± 39.80 <sup>***</sup>	0.56 ± 0.04 <sup>***</sup>	2.88 ± 0.02 <sup>ns</sup>

<sup>#</sup> values are mean ± SE from 6 animals in each group; P values: <sup>a</sup> p≤0.001 vs. vehicle control, <sup>b</sup> p≤0.05 vs. vehicle control, <sup>ns</sup> p>0.05, <sup>\*</sup> p≤0.05, <sup>\*\*</sup> p≤0.01, <sup>\*\*\*</sup> p≤0.001 vs. CCl<sub>4</sub> treated control

**Table 3:** Effect of silymarin and *P. guajava* leaf extract on serum ALT, AST, ALP, bilirubin level and liver weight in thioacetamide (TAA) induced acute liver injury in rats<sup>#</sup>.

Treatment	Dose (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver weight (g/100g bw)
Vehicle control		72.86 ± 6.19	187.16 ± 6.43	400.20 ± 5.85	0.28 ± 0.07	3.12 ± 0.90
CCl <sub>4</sub> control		336.75 ± 32.02 <sup>a</sup>	438.35 ± 10.72 <sup>a</sup>	769.78 ± 23.85 <sup>a</sup>	0.45 ± 0.04 <sup>b</sup>	4.07 ± 0.19 <sup>c</sup>
CCl <sub>4</sub> + Silymarin	100	101.85 ± 4.18 <sup>***</sup>	185.61 ± 8.26 <sup>***</sup>	418.61 ± 5.94 <sup>***</sup>	0.23 ± 0.04 <sup>ns</sup>	3.29 ± 0.10 <sup>*</sup>
CCl <sub>4</sub> + PGJ	250	132.35 ± 7.73 <sup>***</sup>	175.78 ± 7.26 <sup>***</sup>	380.65 ± 13.49 <sup>***</sup>	0.21 ± 0.04 <sup>ns</sup>	3.63 ± 0.18 <sup>ns</sup>
CCl <sub>4</sub> + PGJ	500	32.33 ± 7.74 <sup>***</sup>	237.48 ± 15.39 <sup>***</sup>	479.46 ± 7.52 <sup>***</sup>	0.35 ± 0.09 <sup>ns</sup>	2.76 ± 0.11 <sup>***</sup>

<sup>#</sup> values are mean ± SE from 6 animals in each group; P values: <sup>a</sup> p≤0.001 vs. vehicle control, <sup>b</sup> p≤0.05 vs. vehicle control, <sup>ns</sup> p>0.05, <sup>\*</sup> p≤0.05, <sup>\*\*</sup> p≤0.01, <sup>\*\*\*</sup> p≤0.001 vs. CCl<sub>4</sub> treated control

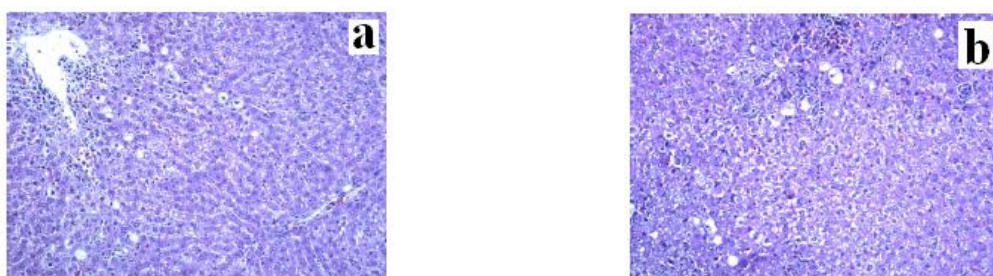
It is known that PCM induces liver injury through the action of its toxic metabolite, N-acetyl-p-benzoquinone-imine, produced by the action of Cytochrome P-450. This metabolite reacts with reduced glutathione (GSH) to yield non-toxic 3-GS-yl-paracetamol. Depletion of GSH causes the remaining quinone to bind to cellular macromolecules leading to

cell death<sup>16</sup>. Damage induced in the liver is accompanied by the increase in the activity of some serum enzymes. The anti-hepatotoxic actions of the extract PGJ (500mg/kg, p.o) was substantiated by significant attenuation of the increased levels of serum enzymes in rats intoxicated with PCM. Drugs having antioxidant activity are also effective in

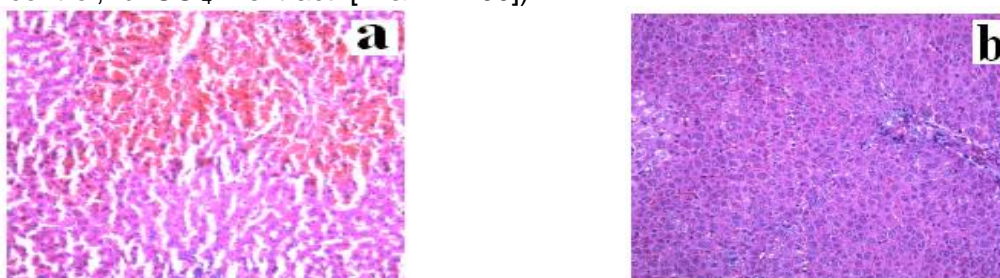
treating paracetamol induced hepatotoxicity by scavenging the free radicals produced by PCM metabolism, thereby preventing the liver induced by both PCM metabolite and due to depletion of glutathione. As mentioned earlier, PGJ is a known antioxidant<sup>7</sup> and this activity may be responsible for its effect in PCM induced hepatotoxic model. The PCM induced a significant increase in liver weight, which is due to the blocking of secretion of hepatic triglycerides into the plasma<sup>15</sup>. PGJ (250 and 500mg/kg, p.o)

prevented the increase in liver weight of rats pretreated with PCM.

TAA interferes with the movement of RNA from the nucleus to the cytoplasm, which may cause membrane injury. A metabolite of TAA (S-oxide) is responsible for hepatic injury<sup>17</sup>. Pretreatment with PGJ (250 and 500mg/kg, p.o) significantly reversed the elevated serum enzyme markers in animals treated with TAA. This effect may also be due to antioxidant effect of PGJ, which may neutralize the reactive metabolite of TAA



**Figure I:** Effect of *P. guajava* leaf extract on acute liver injury induced by CCl<sub>4</sub> (a: CCl<sub>4</sub> treated control, b: CCl<sub>4</sub> + extract [H & E X200])



**Figure II:** Effect of *P. guajava* leaf extract on PCM induced acute liver injury (a: PCM treated control, b: PCM + extract [H & E X200])



**Figure III:** Effect of *P. guajava* leaf extract on TAA induced acute liver damage (a: TAA treated control, b: TAA + extract [H & E X200])

In conclusion, the aqueous extract of *Psidium guajava* Linn. leaves showed good hepatoprotective activity in CCl<sub>4</sub> induced acute liver damage, PCM induced

liver damage and TAA induced liver necrosis. The hepatoprotective activity may be due to the anti-oxidant effect of the plant.

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