

Hepatoprotective activity of *Psidium guajava* Linn. leaf extract

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The study was designed to evaluate the hepatoprotective activity of *P. guajava* in acute experimental liver injury induced by carbon tetrachloride, paracetamol or thioacetamide and chronic liver damage induced by carbon tetrachloride. 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, po) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. The higher dose of the extract (500 mg/kg, po) prevented the increase in liver weight when compared to hepatoxin treated control, while the lower dose was ineffective except in the paracetamol induced liver damage. In the chronic liver injury induced by carbon tetrachloride, the higher dose (500 mg/kg, po) of *P. guajava* leaf extract was found to be more effective than the lower dose (250 mg/kg, po). 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*, Thioacetamide

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 antibacterial¹, antidiarrhoeal², antimycobacterial³, antihyperglycemic⁴, antimalarial⁵, cytotoxic⁶ and antioxidant activities⁷. The antioxidant activity is due to presence of a number of constituents; the major ones are caryophyllene oxide, caryophyllene and a number of tannins⁸. Since, antioxidants 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.

Materials and Methods

Experimental animals — 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 then boiled in a water bath for 1 hr. The boiled leaf extract was then filtered through a muslin cloth. The aqueous extract obtained was evaporated in rotary evaporator to get a powdery mass. The yield was 3.9% (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 procedure⁹.

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Evaluation of hepatoprotective activity — Hepatoprotective activity was evaluated using acute hepatic injury models induced by carbon tetrachloride, paracetamol or thioacetamide. The effect on chronic hepatic injury was evaluated using carbon tetrachloride induced chronic liver damage model.

a. Acute hepatitis models

(i) Carbon tetrachloride (CCl₄) induced acute toxicity: The CCl₄ was diluted with liquid paraffin (1:1) before administration. The animals were divided into 5 groups of 6 each. The animals were then subjected to either one of the following treatments for 9 days.

- Group 1: distilled water (1 ml/kg, po)
- Group 2: distilled water for 9 days + CCl₄ (1 ml/kg, po) on ninth day
- Group 3: silymarin (100 mg/kg/day, po) for 9 days + CCl₄ (1 ml/kg, po) on ninth day
- Group 4: PGJ (250 mg/kg/day, po) for 9 days + CCl₄ (1 ml/kg, po) on ninth day
- Group 5: PGJ (500 mg/kg/day, po) for 9 days + CCl₄ (1 ml/kg, po) on ninth day

Food was withdrawn 12 hr before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 hr after the administration of CCl₄. 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 examination¹⁰.

(ii) Paracetamol (PCM) induced liver toxicity: The same procedure as mentioned above was followed except that the liver was damaged using PCM (1g/kg, po) 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 PCM¹¹.

(iii) Thioacetamide (TAA) induced liver toxicity: The same procedure was followed. Damage was induced by using TAA (100mg/kg, sc), which was prepared in distilled water (2% solution)¹².

b. Chronic toxicity induced by CCl₄

The animals were divided into 5 groups of 6 rats each and treated as follows¹³

- Group 1: distilled water (1 ml/kg, po) for 8 weeks (control).
- Group 2: CCl₄ (1 ml/kg, po) weekly twice for the 8 weeks.
- Group 3: silymarin 100 mg/kg/day, po, for 8 weeks + CCl₄ (1ml/kg, po) weekly twice for 8 weeks.
- Group 4: PGJ (250 mg/kg, po) for 8 weeks + CCl₄ (1 ml/kg, po) weekly twice for 8 weeks.
- Group 5: PGJ (500 mg/kg, po) for 8 weeks + CCl₄ (1 ml/kg, po) weekly twice for 8 weeks.

The animals were sacrificed 24 hr after the last treatment. Blood samples were collected, serum was used for assay of the marker enzymes. The liver was harvested, washed in normal saline, blotted with filter paper and weighed. It was subsequently subjected to histopathological examination.

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 SE and $P \leq 0.05$ was considered significant.

Results

Preliminary 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, po did not produce any mortality. Hence, 1/4th and 1/8th of this dose i.e. 500 mg/kg and 250 mg/kg, po were used.

Carbon tetrachloride induced acute toxicity— The higher dose of PGJ (500 mg/kg po) and silymarin (100 mg/kg; po) produced a significant reduction in serum marker enzymes ($P \leq 0.001$). Lower dose of PGJ (250 mg/kg, po) also produced a significant reduction in ALT, AST, ALP and serum bilirubin when compared to CCl₄ treated group, but it was less effective. Administration of CCl₄ produced a non-significant increase in liver weight. Silymarin and the low dose of PGJ (250 mg/kg, po) did not affect the liver weight, when compared to CCl₄ treated control, whereas higher dose of PGJ (500 mg/kg, po) showed a significant reduction in the liver weight ($P \leq 0.05$) when compared with CCl₄ treated group (Table 1). Histological examination of the liver tissue from CCl₄ treated animals revealed that CCl₄ 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, po) and PGJ (500 mg/kg, po) reduced the inflammation, degenerative changes and steatosis (Fig. 1).

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, po) 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, po) did not show significant effect when compared with the PCM control. Pretreatment with PGJ (250 mg/kg, po) and silymarin significantly reduced the increase in the liver weight seen after PCM intoxication (Table 2). 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 (Fig. 2).

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 500 mg/kg, po) and silymarin significantly reduced the levels of AST, ALT and ALP ($P \leq 0.001$). PGJ at both the dose levels (250 and 500 mg/kg, po) 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 (500 mg/kg, po) and silymarin prevented the increase in liver weight that was observed in TAA treated group, while the lower dose of PGJ (250mg/kg, po) did not produce any significant decrease in liver weight (Table 3). Histological examination showed perlobular hepatocyte necrosis, inflammation and congestion with cytoplasmic vacuolation in TAA

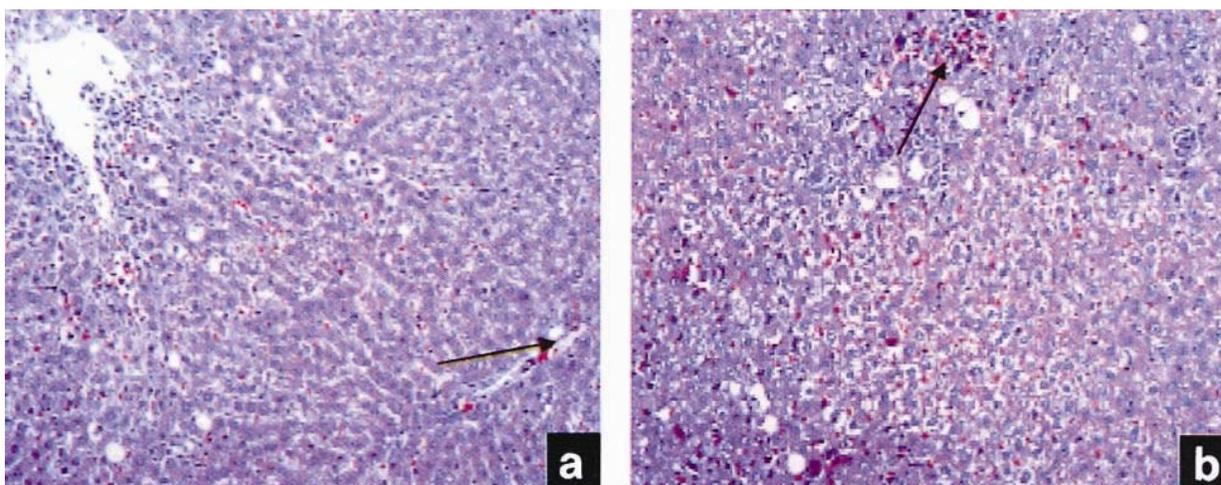


Fig. 1— Effect of *P. guajava* leaf extract on acute liver injury induced by CCl_4 [a: CCl_4 treated control: profound inflammation and congestion, b: CCl_4 + extract : reduced inflammation degenerative changes and steatosis (H & E X200)]

Table 1 — Effect of silymarin and *P. guajava* leaf extract on serum ALT, AST, ALP, bilirubin levels and liver wet weight in CCl_4 induced acute liver injury in rats

Treatment	Dose (po) (mg/kg)	[Values are mean \pm SE from 6 animals in each group]				
		ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver weight (g/100gm body weight)
Vehicle control	--	72.86 \pm 6.19	186.36 \pm 7.81	398.18 \pm 6.73	0.26 \pm 0.08	3.2 \pm 0.09
CCl_4 control	--	384.00 \pm 25.02 ^a	642.20 \pm 31.01 ^a	749.60 \pm 36.25 ^a	1.56 \pm 0.22 ^a	3.78 \pm 0.30 ^b
CCl_4 + Silymarin	100	57.22 \pm 2.81 ^{***}	205.46 \pm 6.12 ^{***}	408.02 \pm 5.58 ^{***}	0.28 \pm 0.03 ^{***}	3.10 \pm 0.06 ^{ns}
CCl_4 + PGJ	250	60.10 \pm 7.70 [*]	572.66 \pm 43.16 ^{ns}	539.96 \pm 79.45 ^{***}	0.26 \pm 0.11 ^{***}	3.18 \pm 0.07 ^{ns}
CCl_4 + PGJ	500	16.72 \pm 4.09 ^{**}	151.74 \pm 13.95 ^{***}	488.82 \pm 10.01 ^{**}	0.26 \pm 0.05 ^{***}	2.99 \pm 0.12 [*]

P values: ^a ≤ 0.001 vs. vehicle control, ^b ≤ 0.05 vs. vehicle control, ^{ns} > 0.05 , ^{*} ≤ 0.05 , ^{**} ≤ 0.01 , ^{***} ≤ 0.001 vs. CCl_4 treated control

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 no necrosis (Fig. 3).

Chronic hepatitis induced by CCl₄— A significant difference in biochemical markers, ALT, AST, ALP and bilirubin was observed between normal and CCl₄ treated group ($P \leq 0.001$). Comparative analysis between different groups revealed that PGJ (500 mg/kg, po) and silymarin (100 mg/kg, po) have similar activity ($P \leq 0.001$), whereas PGJ (250 mg/kg, po) did not prevent the increase in biochemical markers. Pretreatment with PGJ (500 mg/kg; po) and silymarin significantly prevented the increase in liver weight, observed after intoxication with CCl₄. PGJ (250 mg/kg; po) did not produce any significant reduction in liver weight (Table 4). Liver sections from CCl₄ treated control animals showed moderate degree of fatty changes, mild congestion, connective

tissues, proliferation and cirrhosis. It also showed focal areas of coagulating necrosis. Formation of pseudolobular with fibrosin was also observed. Further, there were evidences of regenerating hepatocytes. In silymarin treated animals, there was less amount of necrosis and regeneration. There were mild congestions, mild fatty changes and mild connective tissue proliferation. Animals treated with PGJ (250 mg/kg; po) showed congestion vessels and moderate degree of fatty changes, connective tissue and cirrhosis. The higher dose of PGJ (500 mg/kg; po) reduced the degenerative changes compared to CCl₄ treated animals (Fig. 4).

Discussion

The aqueous extract of *Psidium guajava* leaves showed good hepatoprotective activity when administered at doses of 250 and 500 mg/kg orally and the effect observed was dose dependent. The lower dose (250 mg/kg; po) did not show hepatoprotective effect in chronic hepatic damage

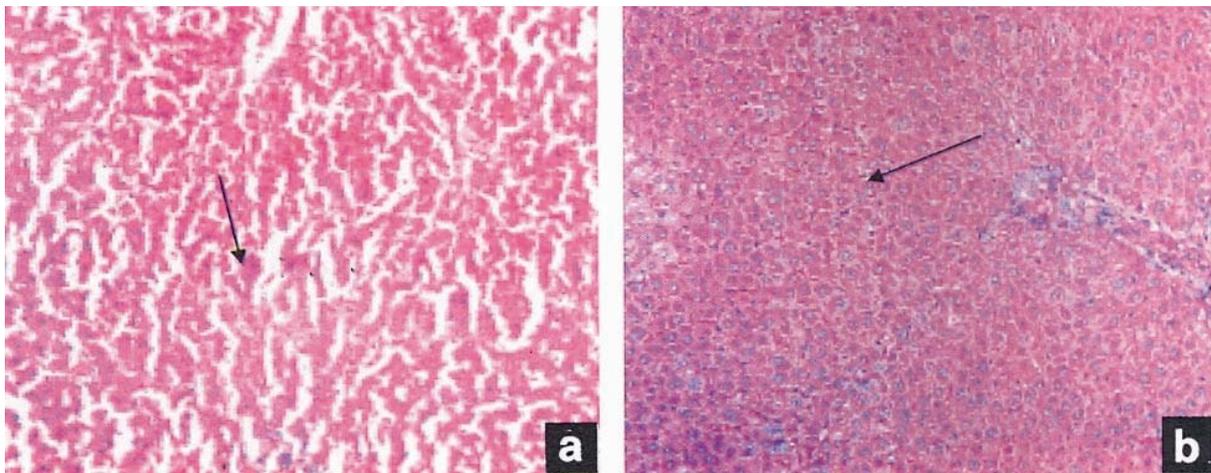


Fig. 2— Effect of *P. guajava* leaf extract on PCM induced acute liver injury [a: PCM treated control: severe congestions, hydropic degeneration, pyknosis and occasional necrosis, b: PCM + extract: mild degeneration and no pyknosis, (H & E X200)]

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.

Treatment	Dose (po) (mg/kg)	[Values are mean \pm SE from 6 animals in each group]				
		ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver wet weight (g/100g body weight)
Vehicle control	-	72.86 \pm 6.19	186.36 \pm 7.81	398.18 \pm 6.73	0.26 \pm 0.08	3.12 \pm 0.09
PCM control	-	384.00 \pm 25.02 ^a	642.20 \pm 31.01 ^a	749.60 \pm 36.38 ^a	1.56 \pm 0.22 ^a	4.40 \pm 0.14 ^a
PCM + Silymarin	100	75.36 \pm 4.25 ^{***}	167.34 \pm 5.51 ^{***}	322.12 \pm 6.94 ^{***}	0.24 \pm 0.05 ^{***}	3.11 \pm 0.05 ^{***}
PCM + PGJ	250	98.28 \pm 2.49 ^{***}	170.50 \pm 5.13 ^{***}	624.28 \pm 88.77 ^{ns}	0.48 \pm 0.02 ^{***}	3.18 \pm 0.07 [*]
PCM+PGJ	500	86.94 \pm 18.94 ^{***}	178.92 \pm 8.07 ^{***}	338.32 \pm 39.80 ^{***}	0.56 \pm 0.04 ^{***}	2.88 \pm 0.02 ^{ns}

P values: ^a ≤ 0.001 vs. vehicle control, ^{ns} > 0.05 , ^{*} ≤ 0.05 , ^{**} ≤ 0.01 , ^{***} ≤ 0.001 vs. PCM treated control.

induced by CCl₄. The effect produced by the higher dose of aqueous extract of *P. guajava* leaves was similar to that produced by silymarin (100 mg/kg; po), a well known hepatoprotective agent.

CCl₄ is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl₄ are largely due to generation of free radicals¹⁴. Drugs having antioxidant activity are effective in treating CCl₄ induced hepatotoxicity. PGJ is known to have antioxidant activity *in vitro*. Different extracts of this plant including the water extract are reported to increase the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH)⁷. The CCl₄ induced a significant increase in liver weight, which is due to blocking of secretion of hepatic triglycerides into the plasma¹⁵. Silymarin and PGJ (250 mg/kg; po) did not prevent the increase of liver weight, whereas PGJ (500 mg/kg; po) prevented the increase of liver weight in rats.

It is known that PCM induces liver injury through the action of its toxic metabolite, N-acetyl-p-benzoquinoneimine, 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 death¹⁶. 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 (500 mg/kg; po) 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 antioxidant⁷ and this activity may be responsible for

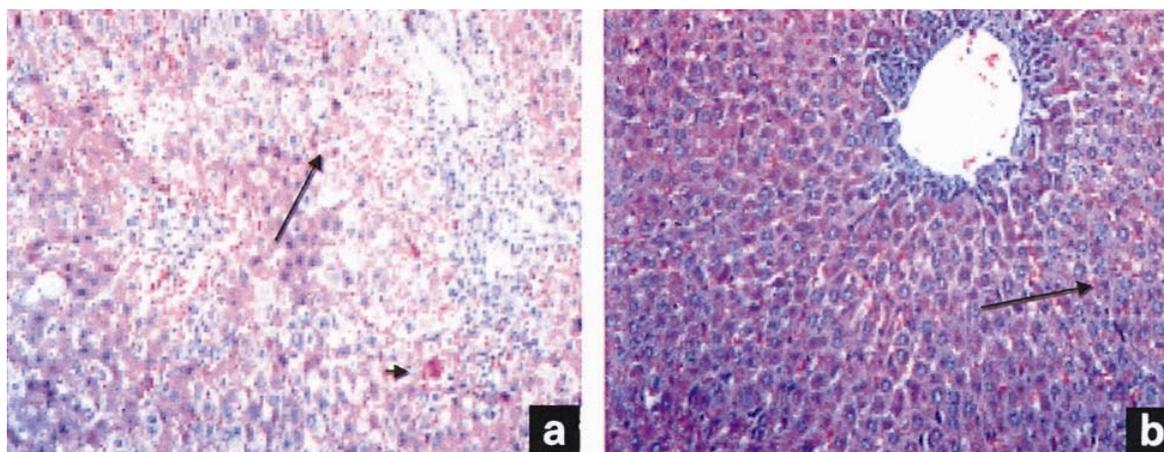


Fig. 3— Effect of *P. guajava* leaf extract on TAA induced acute liver damage [a: TAA treated control: arrow – Peribulbar hepatocyte necrosis, inflammation and congestion; arrow head: cytoplasmic vacuolation, b: TAA + extract: mild inflammation and no necrosis, (H & E X200)]

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.

[Values are mean ± SE from 6 animals in each group]

Treatment	Dose (po) (mg/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver weight (g/100gm body weight)
Vehicle control	-	72.26±5.09	187.16±6.43	400.20±5.85	0.28±0.07	3.12±0.90
TAA control	-	336.75±32.02 ^a	438.35±10.72 ^a	769.78±23.85 ^a	0.45±0.04 ^b	4.07±0.19 ^c
TAA + Silymarin	100	101.85±4.18 ^{***}	185.61±8.26 ^{***}	418.61±5.94 ^{***}	0.23±0.04 ^{ns}	3.29±0.10 [*]
TAA + PGJ	250	132.35±7.73 ^{***}	175.78±7.26 ^{***}	380.65±13.49 ^{***}	0.21±0.04 ^{ns}	3.63±0.18 ^{ns}
TAA + PGJ	500	32.33±7.74 ^{***}	237.48±15.39 ^{***}	479.46±7.52 ^{***}	0.35±0.09 ^{ns}	2.76±0.11 ^{***}

P values: ^a<0.001, ^b>0.05, ^c<0.01 vs. vehicle control, ^{ns}>0.05, * <0.05, ** <0.01, *** <0.001 vs. thioacetamide control.

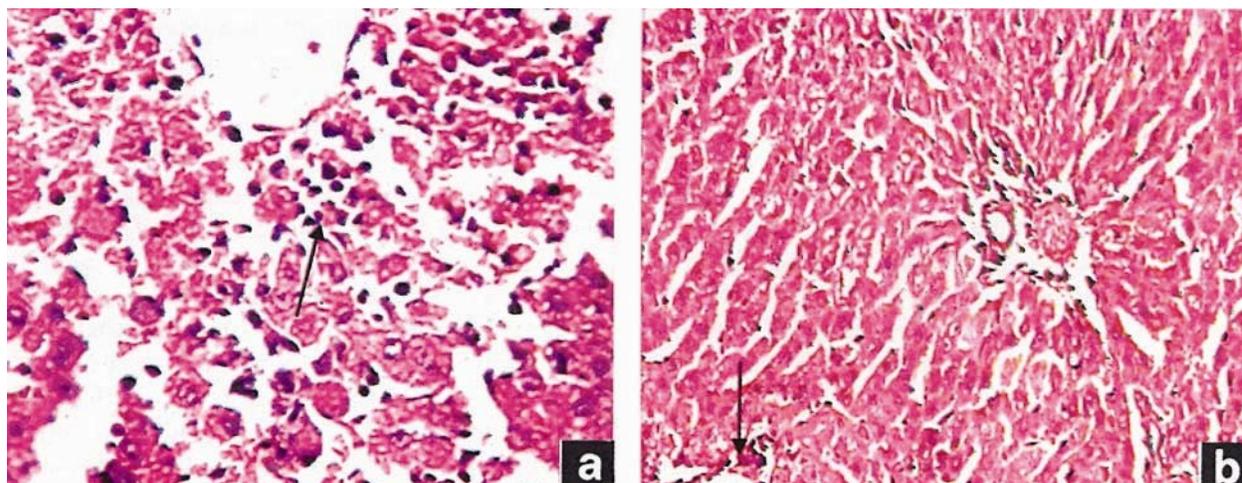


Fig. 4— Effect of *P. guajava* leaf extract on CCl₄ induced chronic liver damage [a: CCl₄ treated control: fatty changes, mild congestion, connective tissue and cirrhosis, b: CCl₄ + extract: degenerative changes (H & E X 200)]

Table 4 — Effect of silymarin and *P. guajava* leaf extract on serum ALT, AST, ALP, bilirubin levels and liver wet weight in CCl₄ induced chronic liver injury in rats.

Treatment	Dose (po.) (mg/kg)	[Values are mean ± SE from 6 animals in each group]				
		ALT (U/L)	AST (U/L)	ALP (U/L)	Serum bilirubin (mg/dl)	Liver wet weight (g/100gm body weight)
Vehicle control	-	49.73 ± 2.03	119.91 ± 4.73	424.86 ± 13.36	0.351 ± 0.020	2.83 ± 0.103
CCl ₄ control	-	320.40 ± 17.04 ^a	617.83 ± 15.42 ^a	755.26 ± 22.78 ^a	0.868 ± 0.038 ^a	3.93 ± 0.197 ^a
CCl ₄ + silymarin	100	137.15 ± 6.59 ^{***}	436.63 ± 18.90 ^{***}	575.46 ± 18.55 ^{***}	0.530 ± 0.014 ^{***}	2.94 ± 0.276 [*]
CCl ₄ + PGJ	250	329.96 ± 8.96 ^{ns}	504.61 ± 26.07 ^{ns}	651.90 ± 25.58 [*]	0.951 ± 0.030 ^{ns}	3.45 ± 0.075 ^{ns}
CCl ₄ + PGJ	500	238.65 ± 15.25 ^{***}	422.23 ± 15.22 ^{***}	613.16 ± 13.51 ^{***}	0.681 ± 0.022 ^{***}	2.87 ± 0.277

P values: ^a≤0.001 vs. vehicle control, ^{ns}>0.05, ^{*}≤0.05, ^{**}≤0.01, ^{***}≤0.001 vs. CCl₄ control

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 plasma¹⁵. PGJ (250 and 500 mg/kg; po) 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 injury¹⁷. Pretreatment with PGJ (250 and 500mg/kg, po) 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.

In conclusion, the aqueous extract of *Psidium guajava* Linn. leaves showed good hepatoprotective activity in CCl₄ induced acute and chronic liver damage, PCM induced liver damage and TAA

induced liver necrosis. The hepatoprotective activity may be due to the antioxidant effect of the plant.

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