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Hepatoprotective activity of *Psidium guajava* extract and its phospholipid complex in paracetamol induced hepatic damage in rats

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Abstract

Psidium guajava is a well known traditional medicinal plant and is used in various indigenous systems of medicine. The present research work aims at evaluating hepatoprotective activity of ethanolic extract of *P. guajava* and the phospholipid complex of the extract with phosphatidylcholine against paracetamol induced hepatic damage in albino rats. The hepatoprotective effect was studied on rat liver damage induced by paracetamol by monitoring serum parameters SGOT, SGPT, ALP, Total protein, Albumin, Globulin, Bilirubin and histopathological alterations. Significant hepatoprotective effects were observed against liver damage induced by paracetamol overdose as evident from decreased serum levels of SGOT, SGPT, ALP and bilirubin in the extract treated groups (200, 400 mg/kg) and phospholipid complex (100mg/kg) compared to the intoxicated controls. The hepatoprotective effect was further verified by histopathology of the liver. The phospholipid complex showed better activity than the plain extracts which was almost comparable to standard silymarin. The aqueous extracts of *P. guajava* and the phospholipid complex exhibited protective effect against paracetamol-induced hepatotoxicity with the complex showing activity better than the plain extract. These results supported the use of this plant for the treatment of hepatitis in oriental traditional medicine.

Keywords: Phospholipid complex, Quercetin, Hepatoprotective, Paracetamol, *Psidium guajava*

Introduction

Psidium guajava L (family Myrtaceae) is a native plant of tropical America. More recent ethnopharmacological studies show that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, for diabetes, hypertension, carries, wounds, pain relief and reducing fever [1]. Leaves of this plant have been reported to contain several compounds such as various flavonoids [2], tannins [3] and terpenoids [4]. Quercetin is the best-known flavonoid from guava leaves [5].

Phosphatidylcholine is a major constituent of cell membranes and it is freely compatible with other nutrients, and when coadministered may enhance their absorption. Several studies have demonstrated that complexation of phospholipids with phytoconstituents increases their bioavailability [6, 7].

Based on its diversified pharmacological properties, an attempt has been made to validate plant for its hepatoprotective potential against hepatotoxin paracetamol in experimental studies.

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The *in vivo* hepatoprotective activity of phospholipid complex was evaluated and compared with plain extract in paracetamol-induced hepatotoxicity in mice model. Its hepatoprotective effect was compared to the effect of standard silymarin which is known to be hepatoprotective against acetaminophen-induced liver damages.

Materials and methods

Plant material

P. guajava (Myrtaceae) fresh tender leaves were collected from a local botanical garden and authenticated at the Department Of Pharmacognosy and Phytochemistry, Prin. K.M.Kundnani College of Pharmacy. The leaves were dried at 40°C in hot air oven for 24 hrs to remove any moisture present.

Preparation of extracts

Dried and powdered leaves of *P.guajava* were extracted by using ethanol in soxhlet apparatus. The total extract obtained was dried at 60 °C on steam bath followed by a vacuum oven (50 °C) to obtain dried extracts. The extractive value was calculated as % w/w yield and was found to be 5.77%.

Preparation of phosphatidylcholine complexes of extract of *P. guajava* and its characterization

The complex was prepared with extract/ quercetin and phospholipid at a molar ratio of 1:2.Weighed amount of extract/ quercetin and phospholipid were taken in a 250 ml round bottom flask and dichloromethane was added. The mixture was refluxed at a temperature not exceeding 60 ◦C for 2 h. The resultant clear solution was evaporated and the resultant mixture was then added to *n*-hexane with continuous stirring. The complex was precipitated and the precipitate was filtered and dried under vacuum to remove traces of solvents. The resultant complex was kept in an amber colored glass bottle, and stored [8].

The phospholipid complex of extract and quercetin were characterized by Nuclear Magnetic Resonance spectroscopy (NMR) and Fourier transform infrared (FTIR) spectroscopy.

The H-NMR spectrums of quercetin, Plant complex and quercetin complex are obtained in DMSO.

For FT-IR, DRS (Diffuse reflectance attachment) is used to measure a diffuse reflection spectrum of a powder sample. The quercetin was analyzed by DRS. KBr Fixed thickness cell is used to measure liquid samples. Thus the phospholipid complex of extract and quercetin was analyzed by preparing solution in dichloromethane.

Experimental animals

Albino Wistar rats (either sex) weighing 200-250 g were obtained from the Glenmark Pharmaceuticals Ltd. The rats were acclimatized to laboratory conditions. They were housed in plastic cages, maintained under 12 h dark light cycle, fed with standard pellet diet and water *ad libitum*. All the experimental procedure and protocols used in the study were approved by IAEC of K.M.K.College of Pharmacy, Cuffe Parade, and as per CPCSEA guidelines. Protocol number is 080910.

Paracetamol induced hepatotoxicity

The model described by Feng-Lin Yen and Tzu-Hui Wu, 2006 [9] was employed with some modifications. Twenty eight rats were randomly divided into seven groups each containing four animals. Group I received saline (10 ml/kg, i.p.) as normal control for seven days; group II received Paracetamol 835mg/kg (in olive oil w/v p.o.) as treated control group; group III received silymarin extract (25 mg/kg, i.p.) as the standard reference; groups IV and V received *Psidium guajava* extract with doses of 200 and 400 mg/kg, respectively, for seven consecutive days; group VI received phospholipid complex at 100 mg/kg p.o., for seven consecutive days and group VII received quercetin at 25 mg/kg p.o., for seven consecutive days. Paracetamol was administered to the animals of Group III, IV, V, VI and VII in a single dose of paracetamol 835mg/kg (in olive oil w/v p.o.) on the seventh day. Animals in all the groups were sacrificed 24 h after paracetamol

administration. The rats were sacrificed on the 8th day under light ether anesthesia. The blood was obtained from all animals by puncturing retro-orbital plexus; serum was separated by centrifugation at 3000 rpm by the pathological centrifuge. The serum parameters SGOT, SGPT, ALP, Total protein, Albumin, Globulin and Bilirubin were estimated. The liver tissue was immediately transferred into 10% formalin for histopathological investigation.

Statistical analysis

The experimental results were expressed as the Mean ± SEM for four animals in each group. The biochemical parameters were analyzed statistically using one-way analysis of variance ANOVA, followed by Dunnett's multiple comparison test (DMCT). P value of < 0.05 was considered as statistically significant**.**

Results

Characterization of phospholipid complex of extract and quercetin:

The formation of a complex is confirmed by a NMR spectroscopy study of the proton, by comparing the spectra of the individual constituents with those of the reaction product (quercetin) In the complex's 1 H-NMR spectrum, the signals of the protons characteristics of the flavonoid molecule are not detectable, or are very enlarged, whereas the protons belonging to the lipid can be observed. Clearly apparent in the same spectrum is a broadening of the band of N-$(Me)_3$ group of choline, showing that this moiety is involved in the complex formation. The complex compound obtained is lipophilic in nature and is soluble in non polar and aprotic solvents.

There is complete disappearance of proton signals in complexes of extract (Fig. 2) and quercetin (Fig. 3) which were present in the quercetin (Fig. 1). Such evidences inferred that the two long aliphatic chains are wrapped around the active principle, producing a lipophilic envelope, which shields the polar head of the phospholipid and the flavonoid molecule.

IR of quercetin showed 3265.49, 3282.84, 3323.35, 3404.36, 3630.03 values representing the OH stretching which confirms the presence of –OH groups, 1664.57 confirms the presence of C=O and 1521.84, 1612.49 values confirm the presence of aromatic structure in quercetin.

IR of phospholipid complex of extract showed 3480.70, 3502.88 values representing the –OH groups which are less as compared to quercetin which confirms the complexation of polyphenols with phospholipid.

IR of Quercetin Phospholipid complex showed 3313.71, 3676.32 values representing the –OH groups which are less as compared to quercetin which confirms the complexation of quercetin with phospholipid.

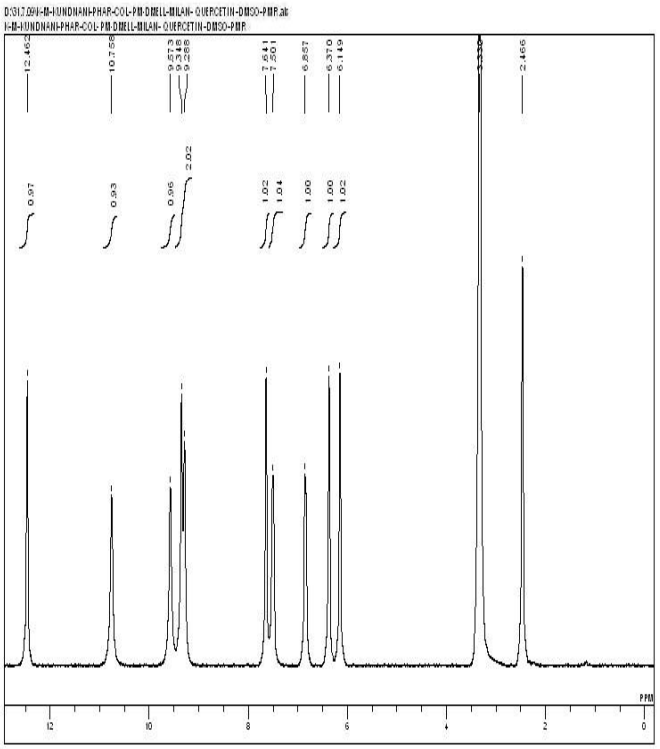

Figure1. Quecetin ^{1}H NMR

Antihepatotoxic activity

A significant elevation in the levels of SGOT, SGPT, ALP and Bilirubin were observed with the administration of the toxicant paracetamol in group II as shown in table 1. The standard and treatment groups *P. guajava* extract 200mg/kg, 400mg/kg and quercetin respectively

demonstrated reduction in the levels of the enzymes. Phospholipid complex showed significant reduction in the levels of enzymes ($p<0.01$) as compared to extract.

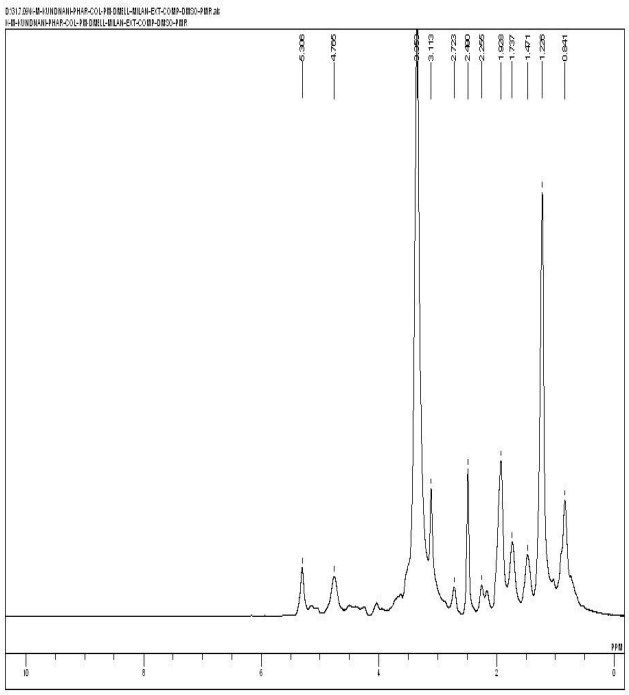

Figure 2. Plant extract phospholipid complex ^{1}H NMR

A significant reduction in the levels of Albumin, Globulin and Total protein were observed with the administration of the toxicant paracetamol in group II. The standard, quercetin and treatment groups *P. guajava* extract 200mg/kg, 400mg/kg respectively demonstrated elevation in the levels of the enzymes. Phospholipid complex showed significant elevation in the levels of above mentioned parameters as compared to extract. Administration of higher dose of the *Psidium guajava* extract 400 mg/kg showed a better hepatoprotective activity than the 200 mg/kg dose indicating dose dependant activity; however the phospholipid complex showed better activity than the extract.

Histopathological studies

The rats treated with a vehicle control (Fig. 7) showed a normal hepatic architecture. The liver samples of paracetamol treated rats showed multifocal moderate to marked degree, marked diffuse granular degeneration, mildly multifocal mild periportal lymphocytic infiltration (Fig. 8). The Histopathological pattern of the livers of the rats treated with paracetamol plus extract 200mg/kg showed moderate diffuse granular degeneration, minimal to mild multifocal, mild lobular disarray (Fig.10) whereas 400 mg/kg dose showed mild diffuse granular degeneration, minimal multifocal periportal lymphocytic infiltration (Fig. 11). However the phospholipid complex treated rat showed multifocal minimal degree, mild diffuse granular degeneration (Fig. 12) demonstrating its potent hepatoprotective effects.

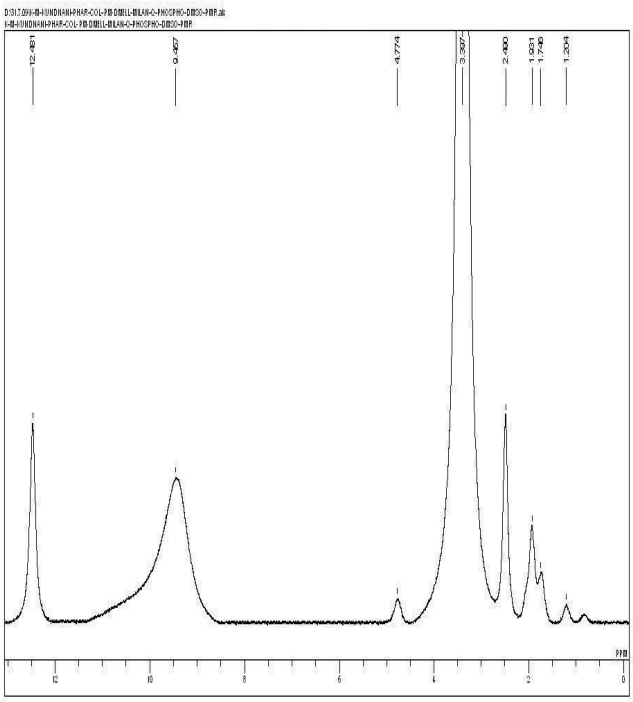

Figure 3. Quercetin phospholipid complex ^{1}H NMR

Discussion

Paracetamol-induced hepatic injuries is the commonly used experimental model for the screening of hepatoprotective drugs [10, 11] and the extent of hepatic damage is assessed by the level of released cytoplasmic enzymes (ALP,

SGOT and SGPT) in circulation [12, 13]. Leakage of cellular enzymes into plasma indicates a hallmark sign of hepatic injury or damage [14, 15].

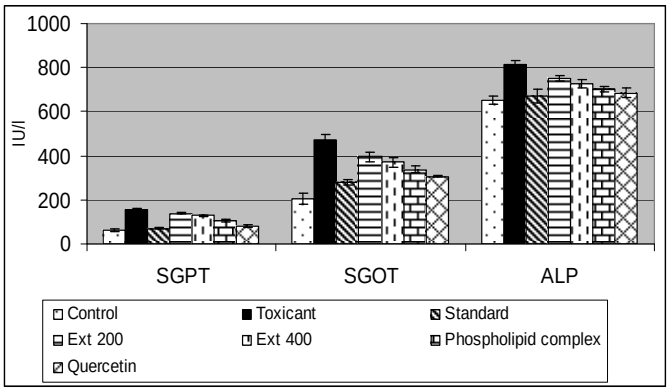

Figure 4. Hepatoprotective action of *Psidium guajava* in rats: serum enzyme activity of SGPT, SGOT and ALP among different treatment groups. Values are mean± S.E.M (*n* = 4)

Paracetamol is mainly metabolized in the liver by glucuronidation and sulfation [16]. However, hepatotoxicity of paracetamol has been attributed to the formation of a toxic reactive metabolite where a part of paracetamol is activated by the hepatic Cytochrome P450 [17].

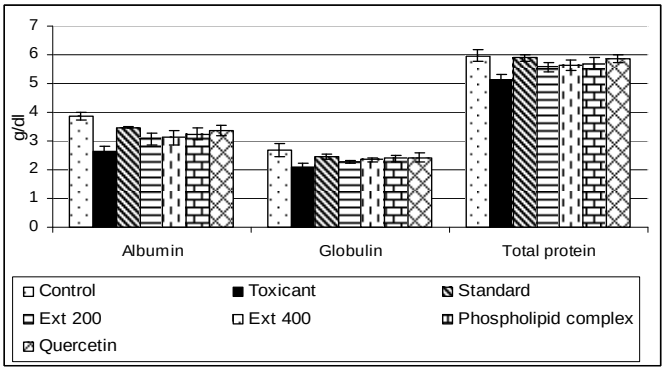

Figure 5. Hepatoprotective action of *Psidium guajava* in rats: serum enzyme activity of Albumin, Globulin and Total protein among different treatment groups. Values are mean± S.E.M (*n* = 4)

This highly reactive metabolite, N-acetyl-p-benzoquinoneimine [18] capable of binding covalently to cellular macromolecules (proteins, DNA) to produce protein adducts.

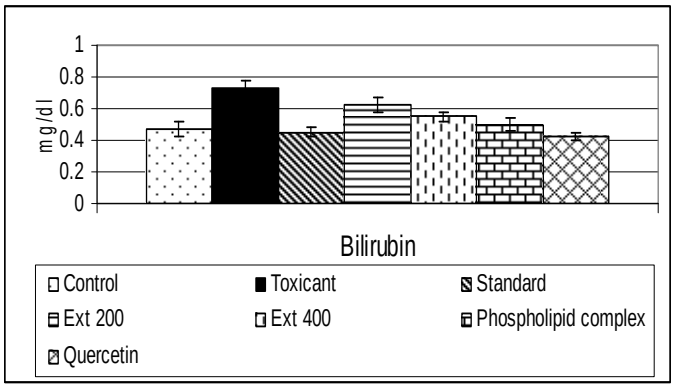

Figure 6. Hepatoprotective action of *Psidium guajava* in rats: serum enzyme activity of Bilirubin among different treatment groups. Values are mean± S.E.M (*n* = 4)

In living systems, liver is considered to be highly sensitive to toxic agents. The study of different enzyme activities such as SGOT, SGPT, ALP, total bilirubin and total protein have been found to be of great value in the assessment of clinical and experimental liver damage [19].

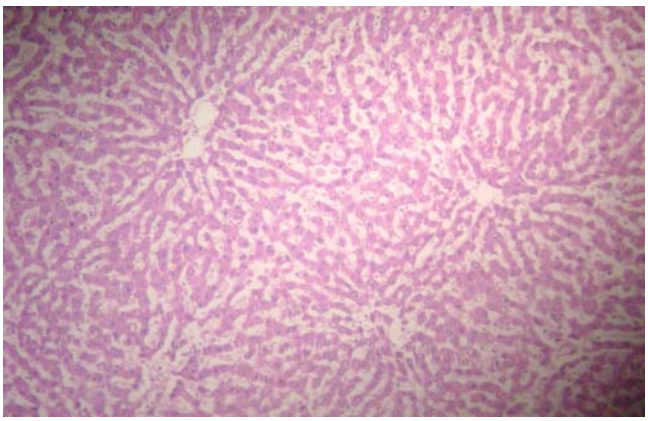

Figure 7. Section of liver of normal control rat showing normal hepatic cells with nuclei and cytoplasm.

Table 1. Effect of the *Psidium guajava* extract on serum parameters in rats intoxicated with paracetamol.

Treatment groups and dose (mg/kg)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	Total Protein g/dl	Albumin g/dl	Globulin g/dl	Bilirubin mg/dl
Group I Control	202.8 ± 13.89**	65 ±4.916**	654.8 ± 16.89**	5.975 ± 0.1887*	3.875 ± 0.125**	2.675 ± 0.2323	0.475 ± 0.0478**
Group II Toxicant control (835 mg/kg)	475 ± 24.71	157.8 ±6.343	814.5 ± 18.2	5.15 ± 0.1555	2.65 ± 0.1756	2.1 ± 0.1354	0.725 ± 0.0478
Group III Standard Silymarin (25 mg/kg)	277 ± 12.55**	70.75 ±3.902**	669.3 ± 30.1	5.9 ± 0.108*	3.475 ± 0.025*	2.45 ± 0.1041	0.45 ± 0.0288**
Group IV Extract (200 mg/kg)	395.8 ± 22.17*	137.3 ±3.75*	751.5 ± 9.717	5.575 ± 0.1493	3.075 ± 0.2136	2.275 ± 0.0629	0.625 ± 0.0478
Group V Extract (400 mg/kg)	370.3 ± 19.5**	128 ±5.307**	729.3 ± 18.2*	5.65 ± 0.1848	3.125 ± 0.2529	2.35 ± 0.0645	0.55 ± 0.0288*
Group VI Phospholipid complex (100 mg /kg)	337.5 ± 13.94**	105.3 ±4.09**	700.8 ± 12.66**	5.7 ± 0.1871	3.25 ± 0.2102	2.4 ± 0.108	0.5 ± 0.0408**
Group VII Quercetin (25 mg/kg)	306.8 ± 5.764**	82.25 ±3.449**	685.3 ± 21.37**	5.875 ± 0.125*	3.375 ± 0.1931	2.425 ± 0.1493	0.425 ± 0.025**

Treatment of the rats with *P. guajava* extracts at 200 mg/kg, 400 mg/kg and phospholipid complex 100mg/kg, orally, for 7 days before paracetamol administration, resulted in a significant reduction of paracetamol-induced elevation of serum marker enzymes and appears to be protective in reducing the injurious effect of paracetamol observed in the study. Silymarin is a known hepatoprotective compound obtained from *Silybum marianum*. It is reported to have a protective effect on plasma membrane of hepatocytes [20].

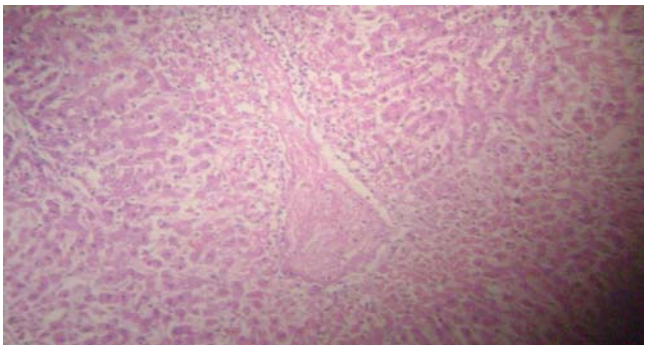

Figure 8. Section of paracetamol overdose-treated rat liver showing Multifocal moderate to marked degree, marked diffuse granular degeneration, mildly multifocal mild periportal lymphocytic infiltration.

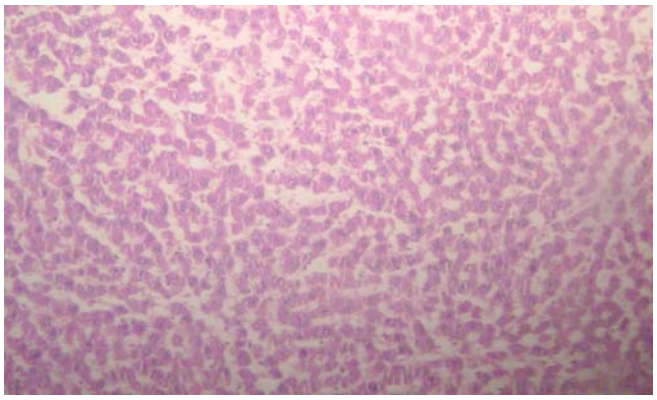

Figure 9. Section of Silymarin (25 mg/kg) + paracetamol overdose-treated rat liver showing mild diffuse granular degeneration.

This is an indication of the stabilization of plasma membrane, as well as repair of hepatic tissue damage caused by paracetamol toxicity.

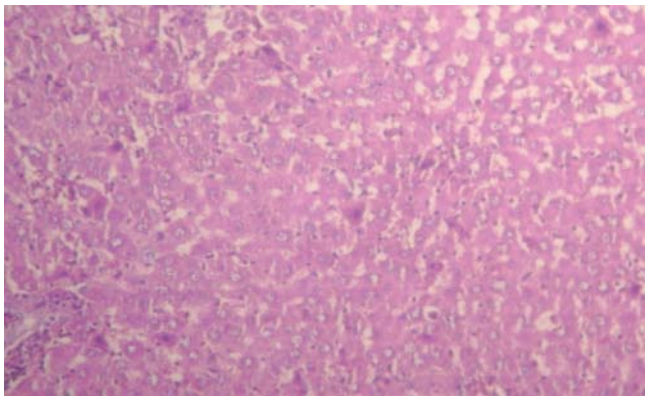

Figure 10. Section of *P. guajava* (200 mg/kg) + paracetamol overdose-treated rat liver showing Moderate diffuse granular degeneration, minimal to mild multifocal, mild lobular disarray.

Prophylactic and curative treatment with extract and phospholipid complex restored all the enzymes studied and bilirubin in a dose dependent manner showing its potential to maintain the normal functional status of the liver. The possible mechanism may be that the antioxidant activity of flavonoids like quercetin present in the extract of *P. guajava* can scavenge free radicals and protect the cell membrane from destruction.

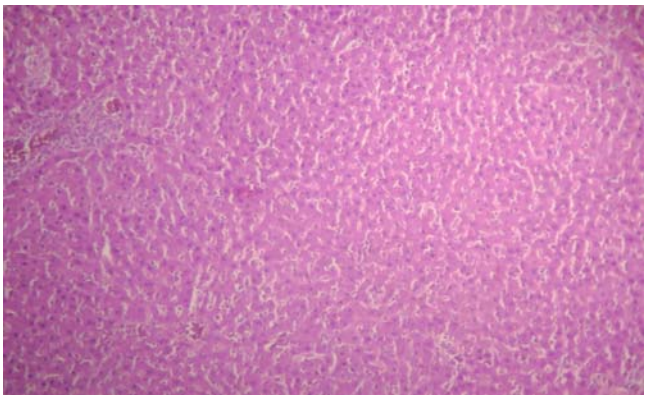

Figure 11. Section of *P. guajava* (400 mg/kg) + paracetamol overdose-treated rat liver showing mild diffuse granular degeneration, minimal multifocal periportal lymphocytic infiltration.

The histopathological observations in paracetamol-treated rats showed severe necrosis, with disappearance of nuclei.

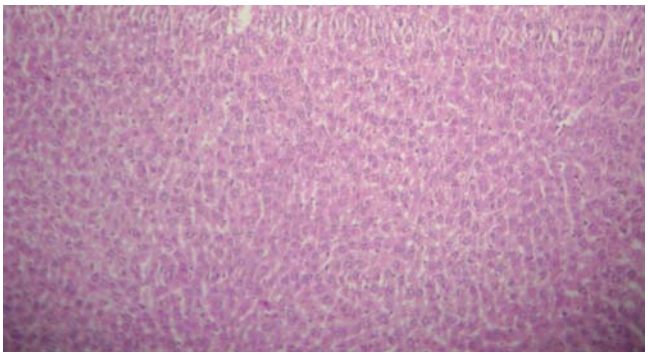

Figure 12. Section of Phospholipid complex (100 mg/kg) + paracetamol overdose-treated rat liver showing multifocal minimal degree, mild diffuse granular degeneration.

This could be due to the formation of highly reactive radicals because of oxidative threat caused by paracetamol. All these changes were very much reduced histopathologically in rats treated with extract and complex.

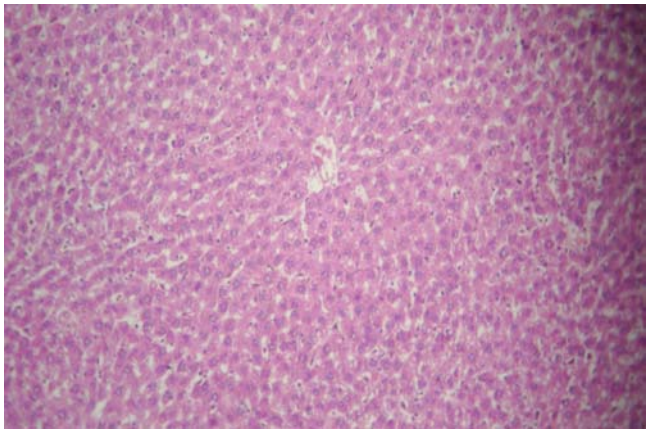

Figure 13. Section of quercetin (25 mg/kg) + paracetamol overdose-treated rat liver showing minimal diffuse granular degeneration.

Based on the above results, it could be concluded that the phospholipid complex exerts significant hepatoprotection than the extract against paracetamol-induced toxicity. The extract showed significant activity against paracetamol induced liver damage in rats in a dose dependant manner. The enhanced activity of phospholipid complex of *P. guajava* could be because of its higher absorption due to complexation with phosphotidyl choline.

Conclusion

In conclusions, the aqueous extracts of *Psidium guajava* and the phospholipid complex exhibited protective effect against paracetamol-induced hepatotoxicity. Further studies are required to isolate the active constituents involved in the hepatoprotective activity of the plant.

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