Hepatoprotective Effect of Indian Medicinal plant *Psidium Guajava* Linn. leaf extract on paracetamol induced liver toxicity in Albino rats

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**ABSTRACT**

The study was designed to evaluate the hepatoprotective activity of *Psidium guajava* (PGJ) in acute experimental liver injury induced by paracetamol (PCM). The effects observed were compared with a known hepatoprotective agent, Silymarin. In the acute liver damage induced by paracetamol, *P. guajava* leaf extracts (500mg/kg, po) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, protein and bilirubin, some antioxidant enzymes, Reduced glutathione (GSH), Glutathione peroxidase (GPx), superoxide dismutase (SOD) and Catalase (CAT) activities, were also evaluated in the rats liver homogenate. The higher dose of the extract (500mg/kg, po) prevented and showed increase in liver weight when compared to hepatoxin treated control, while the lower dose was ineffective in the paracetamol induced liver damage. In the acute liver injury induced by paracetamol the higher dose (500mg/kg, po) of *P. guajava* leaf extract was found to be more effective than the lower dose (250mg/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.

Key words: Paracetamol, Hepatoprotective, *P.guajava*, Silymarin, Antioxidant enzymes.

**INTRODUCTION**

Paracetamol (Acetaminophen), a most commonly used analgesic, it effectively reduces fever and mild-to moderate pain, is considered to be safe at therapeutic doses. However, PCM overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals.[1,2] Most of the experiments aimed to elucidate the mechanism of PCM toxicity were performed on animal model both *in vivo* and *in vitro*.[3,4] When taken at supratherapeutic doses, PCM causes centrilobular hepatocyte degeneration and necrosis in rodents and humans.[5] In response to injury with Paracetamol and other centrilobular hepatotoxicants, there is a recovery phase in which hepatocytes are stimulated to repopulate the liver lobule.[6] Resistance to a different toxicant (heteroprotection) has also been observed.[7] The mechanism(s) underlying the resilience of proliferating hepatocytes to hepatotoxicity is not completely known. A small amount of PCM is metabolized together with cytochrome P450. As a result, N-acetyl-p-benzoquinone imine (NAPQI) or N-acetyl-p-benzoquinimine imine (NAPSQI) appears in the body’s system.[8] Both these compounds are very active chemically and their chemical structures indicate that they are capable of taking part in free radical reactions. Consequently, PCM overdose can lead to a number of unfavorable consequences, especially those affecting the liver.[9,10] A large dose of this drug causes depletion of the cellular glutathione (GSH) level in liver because NAPQI reacts rapidly with glutathione.[11,12] which consequently exacerabtes oxidative stress in conjunction with mitochondrial dysfunction. Thus, the GSH depletion, especially occurring in acute hepatotoxicity, affects liver functions and leads to massive hepatocyte necrosis, liver failure or death. Since oxidative stress and GSH depletion contribute to PCM-induced liver injury; the agent(s) with antioxidant property and/or GSH preserving ability may provide preventive effect against the progression of lipid peroxidation and hepatocellular injury.[13]

Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases.[14] Silymarin extract from the seeds of the plant *Silybum marianum*, also called “milk thistle”. It has been described to be an antioxidant and exhibits anti-carcinogenic, anti-inflammatory, hepatoprotection and growth modulatory effects.[15,16] In this study, Silymarin was used as a positive control against PCM-induced acute hepatic damage in rats.

Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids[17] and Vitamin C[18] etc have received considerable attention in recent years due to their potential benefits to human health. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to human against infection and degeneration diseases.[19] Realizing the fact, this research was carried out to evaluate the antioxidant and hepatoprotective activity of *P.guajava* leaves extract against PCM-induced hepatic damage in rats.

Hepatic injury is not only one of the most common reasons for the termination of drugs in their pre-clinical development but is also a frequent reason for the withdrawal of drugs from the market. Prediction of liver toxicity continues to be a major challenge for the pharmaceutical industry.[20] Preliminary phytochemical investigation of the aqueous extract of the *P.guajava* showed that it contains carbohydrate,tannins, flavonoids, saponins, steroids, proteins, vitamins and amino acids.[21,22] The purpose of this animal study was to examine the preventive effects of Indian plant *P.guajava* on PCM-induced acute hepatic oxidative injury. The hepatoprotective effects of the *P.guajava* was determined by assessing significantly increasing the serum levels of AST, ALT, ALP, bilirubin, and also decrease activity non enzymatic antioxidant like SOD, GPX, CAT and (GSH). In addition, histopathological studies were done to prove its effectiveness in the preventive and curative role against Paracetamol toxicity *in vivo*.

**MATERIALS AND METHODS**

**Animals**

Experimental animals- Wistar albino rats weighing 175-250g used in the present studies were procured from the animal house of Adhiparasakthi College of Arts and Science, Kalavai, Tamil Nadu, India. The animals were housed in polypropylene cages with sterile inert husk materials as bedding.
Table 1. Effect of Indian plant Psidium guajava on serum marker enzymes and total bilirubin level in PCM induced hepatotoxicity in rat.

<table>
<thead>
<tr>
<th>SNo.</th>
<th>Groups</th>
<th>AST (IU/l)</th>
<th>ALT (IU/l)</th>
<th>ALP (IU/l)</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>76.5±2.13</td>
<td>55.4±2.35</td>
<td>63.6±1.09</td>
<td>1.51±0.15</td>
</tr>
<tr>
<td>II</td>
<td>PCM treated</td>
<td>125.7±2.61</td>
<td>84.2±1.84</td>
<td>114.7±3.01</td>
<td>4.8±0.64*</td>
</tr>
<tr>
<td>III</td>
<td>PCM+Silymarin</td>
<td>79.7±1.59</td>
<td>56.9±1.79</td>
<td>70.4±1.48</td>
<td>1.8±0.11*</td>
</tr>
<tr>
<td>IV</td>
<td>PCM+Ps. guajava</td>
<td>77.5±1.56</td>
<td>56.9±1.83</td>
<td>68.4±1.42</td>
<td>1.75±0.05*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>76.5±1.37</td>
<td>55.4±2.35</td>
<td>63.6±1.09</td>
<td>1.51±0.15</td>
</tr>
</tbody>
</table>

Explanations: Each value represents the mean ± SD of six animals. *significant difference at P < 0.05 (BMRT) compared with the control. **significant difference at P < 0.05 (BMRT) compared with the PCM treated group.

All the animals were kept under standard environment condition at 23±2°C (12h light / 12h dark cycle at room temperature) and maintained on commer- cial pellet diet, it was supplied by “HINDUSTAN LEVER” Limited Mumbai, marked under the trade name “Gold mohar” feeds, water was provided ad libitum. The rats were kept in animal house for ten days before starting the experiments.

Plant material

The fresh leaves of Psidium guajava were collected from the campus of Adhiparasakthi College of Arts and Science adjoining areas of G.B.Nagar, Kalavai, Tamil Nadu, India (Voucher No 236 maintained at Adhiparasakthi Agricultural College). The leaves were ground using mortar and pestle using distilled water. For each 100g of crushed leaves 300ml of distilled water was added. The crushed leaves were then boiled in a water bath for 1 hour. The boiled leaf extract was then filtered through a muslin cloth. The aqueous extract obtained was evaporated to get a powdery mass that yielded (4.0% 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 was suspended in water without adding any suspending agent for oral administration.

Experimental design

Paracetamol (PCM) obtained from Indian pharmaceutical company (IPCA) Mumbai. The animals were divided into four groups consisting of six animals each for different experiments. Group I rats served as normal control, Group II (intoxicated group) received orally with a single dose of Paracetamol (1g/kg, po) diluted with sucrose solution (40% w/v). Group III rats were pre-treated with Silymarin commercial drug (100mg/kg, po) for 10 days, followed by rats intoxicated with PCM. Group IV were pre-treated with the P. guajava (500mg/kg, po) for 10 days, followed by rats were intoxicated with PCM. The animals were anesthetized 24 h after the administration of Paracetamol using ether anesthesia. Blood was then drawn by cardiac puncture to determine the serum AST, ALT, ALP, bilirubin activities; finally the animals were then sacrificed. Liver was dissected out for the determination of antioxidant (SOD, GPx, CAT & GSH) status. The liver was then subjected to Histopathological examination.

Serum biochemical assays

At the end of the experiment, the rats were fasted for 24 h prior to the experiments but water was permitted ad libitum. The blood was collected by cardiac puncture from the ether anesthetized rats. The blood was allowed to clot and then centrifuged at 3000 g for 10 min. The haemolysis-free serum samples were stored at -70°C before determination of the biochemical parameters. Serum biochemical parameters AST, ALT, ALP, and total bilirubin were assayed by the method of and  using commercially available kits.

Determination of Antioxidant activity in liver

Preparation of hepatic homogenate

Livers were excised, washed thoroughly in ice-cold saline to remove the blood. They were then gently blotted between the folds of a filter paper and weighed in an analytical balance. Ten percent of homogenate was prepared in 0.05 M phosphate buffer (pH 7) using a polytron homogenizer at 20°C. The homogenate was centrifuged at 3000 g for 20 min to remove the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for further hepatic biochemical assays.

Estimation of reduced glutathione (GSH) level

The glutathione peroxidase (GPx) activity was determined according to the method of with slight modification. The following solutions were pipetted into a cuvette; 0.1 ml of homogenate and 0.8 ml of 100 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, 1 mM NaN₃, 0.2 mM NADPH, 1 unit/ml GSH reductase, and 1 mM GSH. The mixture was pre-incubated for 5 min at room temperature. Thereafter, the overall reaction was initiated by adding 0.1 ml of 2.5 mM H₂O₂. Enzyme activity was calculated by the change of the absorbance at 340 nm for 5 min.

Assay superoxide dismutase activity (SOD)

The activity of superoxide dismutase (SOD) was measured by the method of with modification. About 5 µg of protein-containing liver homogenate were mixed with sodium pyrophosphate buffer, PMT and NBT. The reaction was started by the addition of NADH. Then, the reaction mixture was incubated at 30°C for 90s. Next, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The absorbance of the chromogen formed was measured at 560 nm. One unit of SOD activity is defined as the enzyme concentration required to inhibit chromogen production by 50% in 1 min under the assay condition.

Assay of Catalase (CAT)

The CAT activity was measured in liver homogenates by the method of . For the assay, the liver homogenates containing 5 µg total proteins was mixed separately with 700 µl, 5 mM hydrogen peroxide and incubated at 37°C. The disappearance of peroxide was observed at 240 nm for 15 min. One unit of catalase activity is that which reduces 1 µmol of hydrogen peroxide per minute.

Histopathological Examinations

A portion of the median lobe of the liver was dissected and fixed in 10% neutral buffered formalin solution for 24 h. The remaining livers were stored at -70°C for biochemical analysis. The washed tissue was dehydrated in descending grades of isopropanol and finally cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5 µm thickness after deparaffinized and rehydrated tissues using standard techniques, and the sections were stained with haematoxylin and eosin. The sections were then viewed under light microscope for histopathological changes. The extent of paracetamol induced necrosis was evaluated by assessing morphological changes in liver sections using standard techniques.

Statistical analysis

All data were represented as mean ± SD. Significant difference between the mean values were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison tests (BMRT). The data were analyzed with SPSS version 16 software (SPSS Inc.,
RESULTS

Effect of P.guajava on serum enzymes and total bilirubin levels in PCM induced hepatotoxicity in rat

Serum transaminases, ALP and total bilirubin levels are susceptible to hepatotoxic and serve as markers of liver damage which promotes the release of such serum enzymes from hepatocytes into blood stream. The effects of pretreatment with P.guajava and silymarin on the PCM-induced elevation of serum enzymes AST, ALT, ALP and total bilirubin activities are shown in Table 1. PCM induced elevation were found to be 125.73±2.61 IU L⁻¹ in AST, 84.25±1.84 IU L⁻¹ in ALT and 114.72±3.01 IU L⁻¹ in ALP indicating that PCM have inflicted a significant damage to liver (Table 1). Pre-treatment of P.guajava 1 h prior to PCM administration significantly protected the elevation of transaminases, ALP and total bilirubin activities. The activities of AST, ALT and ALP in the high dose P.guajava plus PCM treated group were 77.58±1.56 IU L⁻¹, 56.93±1.80 IU L⁻¹ and 68.40±1.42 IU L⁻¹, respectively. Similarly, the activity of total bilirubin significantly (P<0.05) decreased in P.guajava plus PCM treated group (1.75±0.05 mg dl⁻¹) in comparison with the PCM induced hepatotoxic group (4.84±0.16 mg dl⁻¹).

Effect of P.guajava on reduced glutathione (GSH) in PCM induced hepatotoxicity in rat

GSH is a crucial antioxidant molecule which is present in cells and is also importantly involved in several metabolic pathways. Fig. 1A shows the effect of P.guajava on the content of GSH in PCM induced hepatotoxicity in rats. The administration of a single dose of PCM (1g/kg, po) to rats resulted in decline of total GSH content in the liver homogenate. Both pre-treatment of P.guajava and silymarin diets significantly inhibited the depletion of GSH (22.30±2.20 and 23.40±1.44 µmol/mg protein respectively), compared to the group of PCM treated rats alone (15.74±1.13 µmol/mg protein). Interestingly, supplementation of P.guajava could protect GSH content depletion induced by PCM.

Effect of P.guajava on glutathione peroxidase (GPx) in PCM induced hepatotoxicity in rat

GPx activity as measured from the liver tissue homogenates of all of the experimental rats are shown in Fig. 1B. The hepatic GPx activity of PCM group were significantly reduced (13.21±1.77 µmol/mg protein) compared to the normal control group (19.71±0.92 µmol/mg protein). However, the GPx activity was significantly increased by intoxication, with pretreatment of P.guajava (17.16±0.84 µmol/mg protein), when compared with the PCM group. In contrast, GPx was influenced by the treatment of PCM, silymarin, or with P.guajava. However, pre-treatment of P.guajava could restore the antioxidant capacity exhausted by PCM.

Effect of P.guajava on superoxide dismutase (SOD) and catalase (CAT) activity

Fig. 1C shows the effects of P.guajava on the hepatic SOD activity in PCM induced oxidative stress in rat. A single dose of PCM resulted in a significant (47.53±0.93 µmol/mg protein) decrease in the activity of SOD, compared with the control group (64.94±1.51 µmol/mg protein). Pre-treatment with P.guajava resulted in a significant increase in the level of SOD (63.81±1.51 µmol/mg protein). Moreover, administration of P.guajava to PCM -treated rats completely reversed the decrease in SOD induced by PCM to control values. CAT activity of the liver tissue homogenates of all of the experimental rats is shown in Fig. 1D. Treatment with PCM resulted in a significant decrease in total CAT levels (14.30±1.01 µmol mg⁻¹ protein) in liver tissues compared with the control group (24.91±1.08 µmol mg⁻¹ protein). Administration of P.guajava to PCM-treated rats resulted in a significantly increase (23.40±1.22 µmol mg⁻¹ protein) in the CAT activity of the liver to normal levels.

Histopathological examination

Liver sections from control rats showed normal lobular architecture and normal hepatic cells with a well-preserved cytoplasm, nucleus and nucleoli were defined (Fig.1A). Whereas rat treated with PCM showed marked regenerative activity in the form of binucleation, prominent nucleoli, nuclear enlargement, loss of nucleus, centrilobular necrosis, and kupffer cells were hyperplastic(Fig 1B). No significant morphological changes were noted in liver of animals given only silymarin, as compared to that of animals in the control group (Fig.1C). Treatment with P.guajava showed normal lobular structure with hardly ascertainable regenerative activity in PCM-challenged animals (Fig.1D).
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paracetamol, CCl

or chemical substances are known to cause hepatic injuries such as they are metabolized into toxic intermediates. Various pharmacological

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Fig 1 (A-D). Hepatoprotective effect of P.guajava on liver. GSH (Fig.1A), GPx (Fig.1B), SOD (Fig.1C) and CAT (Fig.1D) level in PCM induced hepatotoxicity in rat. Each bar represents the mean ± SD of six animals. # P < 0.05 Bonferroni’s multiple comparison tests (BMRT)) significant difference from control. *P < 0.05 (BMRT) significant difference from PCM treated group.

DISCUSSION

In the present study, the Indian medicinal plant P.guajava was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in the serum transaminases, ALP and total bilirubin level in rat induced with PCM hepatotoxicity. Moreover, the P.guajava enhanced the activities of antioxidant enzymes (GSH, GPx, SOD and CAT) against the PCM-induced hepatotoxicity in these animals, suggesting that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effects.

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of the orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. Various pharmacological or chemical substances are known to cause hepatic injuries such as paracetamol, CCl, and dimethylnitrosamine. Excessive dose exposure to these hepatotoxins may induce acute liver injury characterized by abnormality of hepatic function and degeneration, necrosis or apoptosis of hepatocyte. With the increasing ingestion of drugs or exogenous chemicals, the possibility of liver injury will undoubtedly increase. At present, drug or chemical-induced liver injury has become a major clinical problem. Much of attention should be paid to the mechanisms involving drug or chemical-induced liver injury. In addition, the search for effective therapeutical methods for the treatment of drug or chemical-induced liver injury is also very important.

With respect to PCM dependent hepatotoxicity it is generally accepted that P450-dependent bioactivation of PCM is a main cause of potentially fulminating hepatic necrosis upon administration or intake of lethal dose of PCM. PCM hepatotoxicity is the result of a cascade of interrelated biochemical events. In the course of acute liver failure, oxidative stress expressed by oxidant-antioxidant imbalance is profound in liver tissue. In recent studies PCM was found to induce substantial mitochondrial oxidative stress and peroxynitrite formation. This oxidative stress preceded cell injury by several hours and free radical scavengers attenuated PCM induced liver injury.

Serum transaminases (AST & ALT) and ALP activities have long been considered as sensitive indicators of hepatic injury and results indicate increased levels of serum transaminases was noted. ALP has been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage. In the assessment of liver damage by PCM the determination of enzyme levels such as serum transaminases and ALP is largely used. Therefore, the marked release of transaminases and ALT into the circulation indicates severe damage to hepatic tissue membranes during PCM intoxication. In the present study, a single oral dose of PCM at 1g/kg p.o caused a dramatic elevation in the serum transaminases and ALP activities, indicating an acute hepatotoxicity induced by administration of PCM (Table 1). High doses of PCM have been demonstrated to increase the serum levels of AST and ALT in rats. The significantly decreased serum transaminases and ALP activities in the P.guajava and silymarin administered groups prior to PCM demonstrated its hepatoprotective effect.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning. Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility. Bilirubin concentration has been used to evaluate chemically induced hepatic injury. Besides various normal functions liver excretes the breakdown product of hemoglobin namely bilirubin into bile. Pre-treatment of P.guajava prevented severity of liver damage caused by PCM as evidenced by the low level of bilirubin in the serum (Table 1).

Reduced glutathione is a substrate for glutathione related enzymes, and a regenerator for alpha-tocopherol. Therefore, it plays an important role in the antioxidant defense system. It is well known that a large dose of PCM causes hepatic GSH depletion because NAPQI reacts rapidly with glutathione, which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction. The GPx present in the cells can catalyze this reaction. Depletion of GSH below a threshold value was associated with a significant conversion of xanthine dehydrogenase to reversible xanthine oxidase, a superoxide radical generation reaction catalyzing enzyme. In the present study, the hepatic content of GSH and GPx were found to be decreased significantly in PCM intoxicated rats compared with the control rats (Fig.1A and B). However, concomitant administration with P.guajava significantly prevented the PCM induced depletion of hepatic GSH and GPx, indicating the antioxidant effect P.guajava and silymarin in PCM intoxicated rats. All evidence, including serum enzyme activity, GSH level and damage markers show that P.guajava diet could decrease PCM-induced oxidative stress. Living tissues are endowed with innate antioxidant defense mechanisms, such as the presence of the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). A reduction in the activities of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes. Antioxidant enzymes such as SOD and CAT are easily inactivated by lipid peroxides or reactive oxygen species, which results in decreased activities of these enzymes in PCM toxicity. It is most abundant in
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The antioxidant effects of *P. guajava* and silymarin on the PCMI-induced depletion of hepatic antioxidant enzymes are presented in Fig.1C and D. The activities of SOD and CAT in the PCMI group were significantly decreased when compared with the control group. The results strongly suggest that the significant decrease of hepatic CAT and SOD activities observed in rats treated with PCMI may be largely affect due to increased free radical production caused by administration of PCMI [28]. In rats treated with *P. guajava*, however, the activities of these anti-oxidant enzymes were significantly higher than in the rats exposed to PCMI alone. *P. guajava* is similarly rich in phytochemicals and exhibits antioxidant capacity against oxidative stress [37]. Hypopharyngeal gland protein of rat enhances the proliferation of primary cultured rat hepatocytes *in vitro* and this protein was present in *P. guajava* [31]. All evidence, including serum enzyme activity, GSH level and damage markers show that a *P. guajava* diet could decrease PCMI-induced oxidative stress.

Histopathological studies, showed paracetamol to produce extensive vascular degenerative changes and centrilobular necrosis in hepatic tissue. Treatment with silymarin and *P. guajava* extract produced mild degenerative changes and absence of centrilobular necrosis when compared with control.

CONCLUSION

In conclusion, the results of the present study suggest that *P. guajava* has a potent hepatoprotective action upon Paracetamol-induced oxidative stress and liver toxicity in rat. The hepatoprotective effect of *P. guajava* can be correlated directly with its ability to reduce activity of serum enzymes and enhance antioxidant defense status. The findings of this study suggest that *P. guajava* can be used as a safe, cheap, and effective alternative chemopreventive and protective agent in the management of liver diseases.

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