Original Article

Evaluation of Hepatoprotective and Antioxidant activity of *Psidium Guajava* Leaf Extract against Acetaminophen Induced Liver Injury in Rats

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Abstract

The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the aqueous extract of *Psidium guajava* leaf (PGJ) in Wistar albino rats. Protective action of PGJ leaf extract was evaluated using animal model of hepatotoxicity induced by acetaminophen (2 g/kg, bw, p.o.). Liver marker enzymes were assayed in serum and antioxidant status was assessed in liver tissue. Histopathology was also studied. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were increased and the levels of total protein were decreased in acetaminophen treated rats. PGJ leaf at (500 mg/kg, bw, p.o,) doses decreased the elevated levels of all these biochemical parameters and restored the normalcy of total protein significantly. Lipid peroxidation (LPO) was increased significant in liver tissue in the acetaminophen treated rats while the activities of reduced glutathione (GSH), catalase (CAT), glutathione Peroxidase (GPx) and superoxide dismutase (SOD) were decreased. PGJ leaf at the doses of (500 mg/kg, bw, p.o,) decreased the elevated levels of lipid peroxide and restored the normalcy of GPx, GSH, CAT and SOD. Histopathology also shows similar result. *Silymarin* are used in standard for this study. From this study it can be concluded that the PGJ leaf showed significant hepatoprotective and antioxidant action.

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Key words: Acetaminophen, *Psidium guajava*, *Silymarin*, Liver, antioxidant, hepatoprotective

1. Introduction

Liver is the largest metabolic organ of the body and is positioned beneath the diaphragm in the right hypochondrium of the abdominal cavity [1]. Being the major drug-metabolizing and drug detoxifying organ of the body. It is continuously and widely exposed to xenobiotics, hepatotoxins and chemotherapeutic agents that lead to impairment of its functions [2]. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [3]. Hepatotoxicity is one of very common aliment resulting into serious deilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radical. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism [4]. Reactive oxygen species mediated oxidative damage to macromolecules such as lipids, proteins and DNA has been implicated in the pathogenicity of major diseases like cancer, rheumatoid arthritis, degeneration process of aging and cardiovascular disease etc. Antioxidants have been reported to prevent oxidative damage caused by free radicals by
interfering with the oxidation process through radical scavenging and chelating metal ions [5].

Acetaminophen (Paracetamol), a most commonly used analgesics, it effectively reduces fever and mild-to moderate pain, is considered to be safe at therapeutic doses. However, acetaminophen overdose causes severe hepatotoxicity that leads to liver failure in both humans and experimental animals [6,7]. Most of the experiments aimed to elucidate the mechanism of acetaminophen toxicity were performed on animal model both in vivo and in vitro [8,9]. When taken at supratherapeutic doses, acetaminophen causes centrilobular hepatocyte degeneration and necrosis in rodents and humans [10]. In response to injury with Paracetamol and other centrilobular hepatotoxicannts, there is a recovery phase in which hepatocytes are stimulated to repopulate the liver lobule [11]. Resistance to a different toxicant (heteroprotection) has also been observed [12]. The mechanism(s) underlying the resilience of proliferating hepatocytes to further toxicity is not completely known. A small amount of acetaminophen is metabolized together with cytochrome P450. As a result, N-acetyl-p-benzoquinone imine (NAPQI) or Nacetyl-p-benzosemiquinone imine (NAPSQI) appears in the body’s system [13]. Both these compounds are very active chemically and their chemical structures indicate that they are capable of taking part in free radical reactions. Consequently, acetaminophen overdose can lead to a number of unfavorable consequences, especially those affecting the liver [14,15]. A large dose of this drug causes depletion of the cellular glutathione (GSH) level in liver because NAPQI reacts rapidly with glutathione [16,17], which consequently exacerbates 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 contributed paracetamol induced liver injury; the agent(s) with antioxidant property and/or GSH reserving ability may provide preventive effect against the progression of lipid peroxidation and hepatocellular injury [18].

A number of reports indicate that overdose of paracetamol can produce centrilobal hemorrhagic hepatic necrosis in humans and experimental animals [19,20]. Modern medical science does not have, at present, a therapeutic agent which could cure the different liver disorders. In fact; the available remedies are from the traditional system of medicine. Paracetamol has thus been taken as test model to screen the anti-hepatotoxic activity of indigenous drugs. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders [21]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effect. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity [22].

Silymarin has been used for over 20 years in clinical practice for the treatment of toxic liver diseases [23]. Silymarin extract from the seeds of the plant Silybum marianum, also called “milk thistle”. It has been described to be an antioxidant and exhibits anticarcinogenic, antiinflammatory, hepatoprotection and growth modulatory effects [24,25]. In this study, silymarin was used as a positive control to against the paracetamol-induced acute hepatic damage in rats.

Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids [26] and Vitamin C [27] etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [28]. These has been a growing interest in the analysis of certain flavonoids, triterpenoids and steroids stimulated by intense research in 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 [29]. Realizing the fact, this research was carried out to evaluate the antioxidant and hepatoprotective activity of P. guajava leaves extract against acetaminophen-induced hepatic damage in rats.

The present study was to examine the preventive effects of P. guajava (PGJ) on acetaminophen-induced acute hepatic oxidative injury. The hepatoprotective effects of the PGJ was determined by assessing significantly increasing the serum levels of AST, ALT, ALP, bilirubin, tissue LPO activity and also decrease total protein, 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 acetaminophen toxicity in vivo.

2. Materials and Methods

2.1. Drugs and Chemicals

Silymarin was purchased from Vellore, India. Pyridine (C\textsubscript{5}H\textsubscript{5}N), disodium hydrogen phosphate (Na\textsubscript{2}HPO\textsubscript{4}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), dihydrogen potassium phosphate anhydrous (KH\textsubscript{2}PO\textsubscript{4}) and thiobarbituric acid (TBA) were purchased from Merck India Ltd (Mumbai, India). Acetaminophen (APAP), Diagnostic kits for the serum
aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), serum bilirubin, bovine serum albumin (BSA), trichloro acetic acid (TCA), thiobarbituric acid-reacting substances (TABRS), reduced glutathione (GSH), Sodium pyrophosphate, ethylene diamine tetra acetic acid disodium salt (EDTA) 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), β-nicotinamide adenine dinucleotide hydrogen (NADH) were obtained from Sigma Chemical (St. Louis, MO, USA). All other chemicals and reagents were of highest purity commercially available.

2.2. 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. All the animals were kept under standard environment condition at 23±2°C (12h light / 12h dark cycle at room temperature) and maintained on commercial 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.

2.3. Collection and preparation of plant material
The fresh leaves of P. 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 h. 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 PGJ leaves was suspended in water without adding any suspending agent for oral administration.

2.4. Phytochemical evaluation
P. guajava were subjected to qualitative analysis for various phytoconstituent like alkaloids, glycosides, saponins, phytosterols, phenolic compound, tannins, proteins and amino acids [30].

2.5. Experimental design
Acetaminophen (APAP) obtained from Sigma Aldrich, India. 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 acetaminophen (2 g/kg, bw, p.o.) diluted with sucrose solution (40% w/v). Group III rats were pre-treated with Silymarin commercial drug (100 mg/kg, bw, p.o.) for 10 days, followed by rats intoxicated with acetaminophen. Group IV were pre-treated with the PGJ (500 mg/kg, bw, p.o,) for 10 days, followed by rats were intoxicated with acetaminophen. The animals were anesthetized 24 h after the administration of acetaminophen using ether anesthesia. Blood was then drawn by cardiac puncture to determine the serum ALT, AST, ALP, bilirubin activities; finally the animals were then sacrificed. Liver was dissected out for the determination of antioxidant (LPO, SOD, GPx, CAT and GSH) status. The liver was then subjected to histopathological examination.

2.6. Serum biochemical assays
At the end of the experiment, the blood was collected by cardiac puncture from the ether anesthetized rats. The blood sample was allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters namely ALT [31], AST [31], ALP [32], total bilirubin [33] and total protein [34].

2.7. Determination of antioxidant activity in liver
After collection of blood samples the rats in different groups were sacrificed and their livers were excised immediately and washed in ice cold normal saline, followed by 0.15M Tris-Hel (pH 7.4) blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation [35]. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione [36]. The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40°C. The supernatant thus obtained was used for estimation of SOD [37], CAT [38] and GPx [39] activities.

2.8. Histopathological examination
Liver pieces were preserved in 10% formaldehyde solution. The pieces of liver processed and embedded in paraffin wax. Sections of about 4-6 microns were made and stained with hematoxylin and eosin and photographed.

2.9. Statistical analysis
The results were expressed as mean ± SEM and were analyzed for statistically significant difference using one-way ANOVA followed by Bonferroni’s multiple comparison tests (BMCT) post hock test. The data were analyzed with SPSS version 16 software (SPSS Inc., Chicago, USA). The difference showing a level of p < 0.05 was considered to be statistically significant.

3. Results
3.1. Preliminary phytochemical investigation
The preliminary phytochemical investigation of the PGJ leaves showed moderate presence of flavanoids and very highly present of saponins, phytosterols, phenolic compound, tannins.

3.2. Serum biochemical parameters
The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total bilirubin were significantly (P<0.05) increased
and the levels of total protein were significantly (P<0.001) decreased in acetaminophen treated rats when compared to control group (Table 1). Administering Silymarin and PGJ leaves (100 mg/kg, bw, p.o, and 500 mg/kg, bw, p.o, respectively) reduced the elevated levels of AST, ALT ALP and total bilirubin levels as well as restore the levels of total protein towards normalcy when compared to acetaminophen treated rat.

3.3. Hepatic oxidative stress parameters

Lipid peroxidase (LPO) level was significantly (P<0.001) increased and the levels of GSH, CAT and SOD were significantly (P<0.001) decreased in acetaminophen treated rats when compared to control group. Administering Silymarin and PGJ leaves (100 mg/kg, bw, p.o, and 500 mg/kg, bw, p.o, respectively) significantly (P<0.05) decreased the elevated levels of Lipid peroxidase (LPO) content as well as increased significantly (P<0.001) the antioxidant levels (Table 2).

3.4. 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 rats treated with acetaminophen 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 Psidium guajava showed normal lobular structure with hardly ascertainable regenerative activity in acetaminophen challenged animals (Fig.1D).

4. Discussion

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 acetaminophen, CCl₄ 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 hepatocytes [40]. With the increasing ingestion of drugs or exogenous chemicals, the possibilities of liver injury will undoubtedly increase [41]. At present, drug or chemical-induced liver injury has become a major clinical problem. Much 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 [14].

With respect to acetaminophen dependent hepatotoxicity it is generally accepted that P450-dependent bioactivation of acetaminophen is a main cause of potentially fulminant hepatic necrosis upon administration or intake of lethal dose of acetaminophen [42,16]. Acetaminophen hepatotoxicity is the result of a cascade of interrelated
biochemical events [43]. In the course of acute liver failure, oxidative stress expressed by oxidant-antioxidant imbalance is profound in liver tissue. In recent studies acetaminophen was found to induce substantial mitochondrial oxidative stress and peroxynitrite formation [44]. This oxidative stress preceded cell injury by several hours [45] and free radical scavenger’s attenuated acetaminophen induced liver injury [9]. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. Hepatocellular necrosis or membrane damage leads to very high levels of serum AST and ALT released from liver to circulation. Among the two, GPT is a better index of liver injury, since ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner, thus liver ALT represents 90% of total enzyme present in the body [46].

The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver [47]. ALP activities on the other hand are related to functioning of hepatocytes, its increase in serum is due to increased synthesis in the presence of increased biliary pressure [48]. In the present study, Treatment with PGJ leaf (500 mg/kg, bw, p.o,) suppressed the elevated serum levels of AST, ALT towards the respective normal value this clearly indicates that the plant extract has stabilizes the plasma membrane as well as helped in healing of the hepatic tissue damage.

Serum ALP and total bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure [49]. The PGJ leaf (500 mg/kg, bw, p.o,) is able to improve the secretory mechanism of hepatic cells and reduces the elevated levels of ALP and total bilirubin. The site specific oxidative damage of some of the susceptible amino acids of proteins is regarded as the major cause of metabolic dysfunction during pathogenesis [50]. Hypoalbuminemia is most frequent in the presence of advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells [51]. The lowered level of total proteins recorded in the serum as well as liver of acetaminophen treated rats reveals the severity of hepatopathy. PGJ leaf has failed to restore the normalcy of total protein level.

Lipid peroxidation has been postulated to be the destructive process in liver injury due to CCl₄ administration [52]. The increase in LPO levels content suggest enhancement of lipid peroxidation which is leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals [53,54]. Treatment with PGJ leaves did not reduced the levels of lipid peroxidation.

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 [55]. It is well known that a large dose of acetaminophen causes hepatic GSH depletion because NAPQI reacts rapidly with glutathione [17], which consequently exacerbates oxidative stress in conjunction with mitochondrial dysfunction. The GPx present in the cells can catalyze this reaction. Cighetti et al., 1993 [56] reported that depletion of GSH below a threshold value was associated

Table 1. Effect of the Psidium guajava leaves aqueous extract (PGJ) on biochemical parameters in acetaminophen-induced hepatic injury in rats

<table>
<thead>
<tr>
<th>S.No</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>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>75.44±1.29</td>
<td>56.39±2.73</td>
<td>62.14±2.83</td>
<td>1.55±0.11</td>
<td>7.03±0.42</td>
</tr>
<tr>
<td>II</td>
<td>Acetaminophen (2g/kg, bw, p.o.)</td>
<td>120.73±2.61#</td>
<td>80.28±2.44#</td>
<td>115.32±4.25#</td>
<td>4.29±0.83#</td>
<td>4.82±0.40#</td>
</tr>
<tr>
<td>III</td>
<td>Acetaminophen (2g/kg, bw, p.o.)</td>
<td>78.30±1.28*</td>
<td>58.65±1.97*</td>
<td>74.34±2.03*</td>
<td>1.73±0.01*</td>
<td>6.57±0.03*</td>
</tr>
<tr>
<td></td>
<td>+ Silymarin (100mg/kg, bw, p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Acetaminophen (2g/kg, bw, p.o.)</td>
<td>77.23±2.81**</td>
<td>57.33±2.93**</td>
<td>67.30±3.21**</td>
<td>1.74±0.05**</td>
<td>6.77±0.27**</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of six animals, one way analysis of variance (ANOVA) followed by (BMCT) post hock analysis. *significant difference at P<0.001 compared with the control. Significant difference at *P < 0.05 and **P< 0.001 (BMCT) compared with the acetaminophen treated group.
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 acetaminophen intoxicated rats compared with the control rats. However, concomitant administration with honey significantly prevented the acetaminophen induced depletion of hepatic GSH and GPx, indicating the antioxidant effect honey in acetaminophen intoxicated rats.

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 [57]. 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 acetaminophen toxicity. It is most abundant in the liver and is responsible for the catalytic decomposition of H$_2$O$_2$ to oxygen and water [58]. SOD is an extremely effective antioxidant enzyme, and is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to H$_2$O$_2$ [59].

The activities of SOD and CAT in the acetaminophen 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 acetaminophen may be largely affect due to increased free radical production caused by administration of acetaminophen [59]. In rats treated with PGJ, however, the activities of these antioxidant enzymes were significantly higher than in the rats exposed to acetaminophen alone. PGJ is similarly rich in phytochemical and exhibits antioxidant capacity against oxidative stress [60]. Hypopharyngeal gland protein of rat enhances the proliferation of primary cultured rat hepatocytes in vitro and this protein was present in PGJ [29]. All evidence, including serum enzyme activity, GSH level and damage markers show that a PGJ diet could decrease acetaminophen-induced oxidative stress.

Histopathological studies, showed paracetamol to produce extensive vascular degenerative changes and centrlobular necrosis in hepatocytes. Treatment with silymarin and PGJ extract produced mild degenerative changes and absence of centrlobular necrosis when compared with control.

### Table 2. Effect of the *Psidium guajava* leaves aqueous extract (PGJ) on antioxidant activity in acetaminophen-induced hepatic injury in rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>LPO (nmol/mg protein)</th>
<th>GSH (µmol/mg protein)</th>
<th>SOD (µmol/mg protein)</th>
<th>CAT (µmol/mg protein)</th>
<th>GPx (µmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Normal control</td>
<td>1.75±0.02</td>
<td>24.74±2.29</td>
<td>65.03±1.92</td>
<td>25.03±1.92</td>
<td>20.90±1.24</td>
</tr>
<tr>
<td>II.</td>
<td>Acetaminophen (2g/kg, bw, p.o.)</td>
<td>6.70±0.25*</td>
<td>15.20±1.20*</td>
<td>48.09±1.22*</td>
<td>14.20±1.09*</td>
<td>13.25±1.09*</td>
</tr>
<tr>
<td>III.</td>
<td>Acetaminophen (2g/kg, bw, p.o.) + Silymarin (100mg/kg, bw, p.o.)</td>
<td>2.04±0.01*</td>
<td>22.35±1.03*</td>
<td>62.30±2.41*</td>
<td>22.35±1.92*</td>
<td>17.33±1.58*</td>
</tr>
<tr>
<td>IV.</td>
<td>Acetaminophen (2g/kg, bw, p.o.) + PGJ (500mg/kg, bw, p.o.)</td>
<td>1.92±0.02**</td>
<td>23.44±1.98**</td>
<td>63.40±2.05**</td>
<td>24.22±1.90**</td>
<td>18.46±1.74**</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SE of six animals, one way analysis of variance (ANOVA) followed by (BMCT) post hock analysis. *significant difference at P<0.001 compared with the control. Significant difference at *P < 0.05 and **P< 0.001 (BMCT) compared with the acetaminophen treated group.

5. Conclusion

In conclusion, the results of this study demonstrate that *Psidium guajava* has a potent hepatoprotective action upon acetaminophen-induced oxidative stress and liver toxicity in rat. The hepatoprotective effect of *Psidium guajava* can be correlated directly with its ability to reduce activity of serum enzymes and enhance antioxidant defiance status. The findings of this study suggest that *Psidium 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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