Bioactivity of *Platycerium angolense* Flavonoid Fraction on Biochemical Parameters of Acetaminophen-induced Rats

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This study evaluated the effect of flavonoid fraction (FF) of *Platycerium angolense* on acetaminophen-induced liver damage in rat with a view to considering the possibility of utilizing the extracts of the plant for the management and treatment of liver related ailments. Powdered leaves of *P. angolense* were extracted with methanol 70% (v/v) to yield methanolic extract of *P. angolense* (MEPA). The MEPA followed successive partitioning with n-hexane, chloroform and ethylacetate. The ethylacetate fraction was termed flavonoid fraction (FF). FF was phytochemically screened and total phenolic and flavonoid contents estimated. Biochemical effects of FF on liver marker enzymes (L-alanine and L-aspartate aminotransferases, gamma glutamyltransferase, acid and alkaline phosphatases); lipid profiles (cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol and very low density lipoprotein (VLDL)-cholesterol) enzymatic and non-enzymatic antioxidants (catalase, superoxide dismutase, peroxidase, glutathione (GSH), lipid peroxidation) and metabolites (total protein, albumin and bilirubin) were evaluated in the plasma and liver homogenates of albino rats. Phytochemical screening of FF tested positive for the presence of flavonoids, saponins, anthraquinones and cardiac glycosides. The total phenolics and flavonoids in FF were (33.58 ± 0.11 mg TAE/g, 50.98 ± 0.89 mg RE/g), respectively. Analyses of plasma and liver homogenates of rats (control and experimental) revealed that acetaminophen caused metabolic dysfunction in acetaminophen-induced rats. Administration of FF reversed the altered biochemical parameters. The study concluded that FF of *P. angolense* increases plasma lipid profile level, exhibits moderate anti-oxidant activity and elicits hepatoprotective potential.

**Key words:** Hepatotoxicity, *Platycerium angolense*, acetaminophen, flavonoids, flavonoid fraction.

INTRODUCTION

Flavonoids belong to a group of polyphenolic compounds with diverse chemical structures and properties. They are found in fruits, nuts, seeds, vegetables, grains, bark, roots, stems, flowers of plants as well as in tea and wine (Middleton, 1998) and constitutes integral part of human diets (Agati and Tattini, 2010; Pollastri and Tattini, 2011). They are powerful antioxidants against free radicals and are described as radical scavengers (Pal et al., 2009), the
activity that is attributed to their hydrogen donating ability. The phenolic groups of flavonoids serve as a source of a readily available “H” atoms such that the subsequent radicals produced may be delocalized over the flavonoid structure (Tripoli et al., 2007; Heim et al., 2002). Flavonoids have been reported to exert wide range of biological activities which include anti-inflammatory, antibacterial, antiviral, antiallergic (Cushnie and Lamb, 2005) cytotoxicity, antitumour, treatment of neuroprotective, vasodilatory action (Tsuchiya, 2010; Chebil et al., 2006). Flavonoids and other phenolics have exhibited their effects as antioxidants, free radical scavengers and chelators of divalent cations (Cook and Shamman, 1996; Agati et al., 2012). In addition, flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities (Chebil et al., 2006).

Hepatotoxicity may be defined as injury to the liver that is associated with impaired liver functions caused by exposure to drugs or other infectious agents (Navarro and Senior, 2006). Acetaminophen (AAP) is a safe and effective analgesic/antipyretic drug when used at therapeutic levels (Rumack, 2004). However, an acute or cumulative overdose causes severe liver injury with the potential to progress to liver failure (Lee, 2004). Acetaminophen is extensively metabolized by conjugation with sulphate and glucuronic acid when a normal dose is used. N- acetyl - p- benzo- quinoneimine (NAPQI) a highly electrophilic metabolite that triggers liver damage is generated when a small fraction of the drug is subjected to oxidation reactions catalyzed by Cytochrome P450 enzymes in the liver (Chen et al., 2009). Normally, toxic oxidation metabolites generated in the liver are converted into non-toxic metabolites excreted in urine via conjugation with glutathione (GSH) containing sulphydryl groups. However, high doses of paracetamol limit the ability of GSH to detoxify NAPQI, and results in the consumption of liver GSH stores (Mitchell et al., 1973; Savides and Oehme, 1983).

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Platycerium (Platycerium angolense Welw; Polypodiaceae family) is an epiphytic genus that grows naturally on branches and trunks of trees. It is native to tropical and temperate areas of South America, Africa, Southeast Asia, Australia and New Guinea. Bode and Oyedapo (2011) earlier reported that studies on the biological activity of the extract of P. angolense revealed that the plant contains bioactive molecules which exhibited potent and appreciable antioxidant and anti-inflammatory activities. This study was designed to evaluate the effect of flavonoid fraction of P. angolense against acetaminophen-induced liver damage in rats with a view of investigating further the activity of P. angolense and the possibility of using this extract for the management and treatment of liver-related disorders.

MATERIALS AND METHODS

Collection, identification and preparation of plant materials

Fresh leaves of P. angolense, Welw. Ex Hook, were collected from Azadirachta indica, (Neem) tree, Opposite Staff Club, OAU, Staff Quarters at the main campus of Obafemi Awolowo University, Ile-Ife, Nigeria. The plant was identified and authenticated by Dr. F. A. Oloyede at the IFE Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. The specimen sample was deposited at IFE Herbarium where specimen identification number (IFE 17340) was collected. Fresh leaves of P. angolense were cut into bits, air-dried in the laboratory over a period of 4 weeks and pulverized into powder using hand operated machine.

Reagents and chemicals

All the reagents and chemicals used in this study were of analytical grade, and were procured from British Drug House (BDH) Chemicals Limited, Poole, England. Diagnostic kits, for lipid profile, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase, total protein, albumin and bilirubin assays; were procured from Randox Laboratories Ltd, United Kingdom. Silymarin was obtained from Sigma Chemical Company and Acetaminophen was obtained from Drug Research and Production Unit (DRPU), Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife.

Experimental animals

Thirty (30) healthy Albino rats were purchased from Empire Farms, Osogbo, Osun State, Nigeria. The rats were acclimatized for two weeks and fed with Standard Rat C (Empire Vacuum) diet. The experiment was carried out in the Department of Biological Sciences, Obafemi Awolowo University, Osogbo, Nigeria.

Preparation of methanolic extract (ME) and flavonoid fraction (FF) of P. angolense

The methanolic extract of P. angolense was prepared according to a modified method of Oyedapo and Amos (1997). Powdered plant (500 g) was macerated with 6 L of 70% (v/v) methanol for 24 h. The suspension was filtered through two layers of white cotton-cloth and the residue was re-extracted with same solvent for another 24 h. The filtrates were combined and centrifuged at 3,000 rpm for 10 min at 25°C, on a Table Centrifuge (Model 90-2 Microfield Instrument, Essex, England).

The supernatant was evaporated to dryness at 40°C under reduced pressure on Edward High Vacuum Pump, Model ED-100 (Edward Vacuum Components, Crawley, England). The brown residue termed methanolic extract (ME) was collected and kept in
desiccator until required for analysis. Methanolic extract (50 g) was taken up in warm water (250 ml) until totally dissolved and filtered. The filtrate was partitioned successively with *n*-hexane (10 × 250 ml), chloroform (10 × 150 ml) and ethyl acetate (10 × 200 ml). The solvent and filtrate were shaken together and poured into the separating funnel, allowed to separate properly and each fraction was collected appropriately. This process was followed with other solvents. The fractions from the same solvent were pooled together and evaporated at reduced temperature (40°C), as earlier described. The residue from each fraction after evaporation was kept in a desiccator until required for analysis.

Phytochemical screening of fractions

The fractions (*n*-hexane, nHF, chloroform, CF, ethyl acetate, EAF and aqueous, AqF) were phytochemically screened using standard procedures earlier described by Sofowora (2002) and Oyedapo et al. (1999).

Estimation of total phenolic and flavonoid contents of fractions

The total phenolic contents of the fractions were carried out according to the method of Singleton et al. (1999). The reaction mixtures contained 0.2 ml (1 mg/ml stock) of the fraction, and Folin Ciocalteu’s (phenol) reagent (1.5 ml, 1:10). 10% (w/v) NaHCO₃ (1.5 ml) was added. The mixture was further incubated for 1 h 30 min in the dark. The absorbance was read at 725 nm against the reagent blank. The standard calibration curve was prepared with tannic acid (0.02 mg/ml). The assays were carried out in triplicate and the mean of three readings was estimated. The concentrations of the phenolics were obtained from standard calibration curve by extrapolation and expressed as milligram tannic acid equivalent per g of extract (mg TAE/g extract) (Figure 1). The total flavonoid contents of the fractions were carried out according to the method of Singh et al. (2010). The reaction mixtures consisted of 0.5 ml of the fraction (1 mg/ml), distilled water (1.5 ml), 5% (w/v) NaNO₂ (0.3 ml), 10% (w/v) AlCl₃ (0.3 ml), and 4% (w/v) NaOH (2.0 ml). The reaction mixture was incubated at 25°C for 10 min after which the absorbance was read at 500 nm against the reagent blank. The assay was carried out in triplicates and the mean was obtained. Standard calibration curve was prepared by using rutin (0.1 mg/ml). The concentrations of the flavonoids were obtained from standard calibration curve and expressed as milligram rutin equivalent per g of extract (mg RE/g extract) (Figure 2).

Grouping and treatment of experimental animals

Albino rats (30) were divided into 6 groups of 5 animals per group and treated as follows:

- **Group 1**: Rats served as control and received distilled water.
- **Group 2**: Rats were administered acetaminophen 150 mg/kg body weight (bwt).
- **Group 3**: Rats were treated with standard drug (silymarin 50 mg/kg bwt) only.
- **Group 4**: Rats were administered acetaminophen (150 mg/kg bwt) and silymarin 50 mg/kg bwt.
- **Group 5**: Rats were pretreated with flavonoid fraction (125 mg/kg bwt) and administered with acetaminophen 150 mg/kg bwt 1 hour later.
- **Group 6**: Rats were administered acetaminophen (150 mg/kg bwt) and treated with flavonoid fraction 125 mg/kg bwt an hour later. The rats were orally treated once daily consistently for 15 days.

Sacrificing of experimental animals

The rats were sacrificed under slight ether anaesthesia on the 16th day. The rats were dissected; blood was collected by cardiac puncture, into an anticoagulant bottle containing (3.8% (w/v) trisodium citrate). Also, livers were aseptically collected, perfused in normal saline (0.85% (w/v) NaCl) and blotted on tissue paper. The livers were stored frozen until required for analysis.

Preparation of blood plasma

The blood samples were centrifuged at 3,000 rpm for 10 min on a Bench centrifuge (Model 90-2, Microfield Instrument, Essex, England) at room temperature. The plasma was carefully collected, stored frozen and used for biochemical analyses.

Preparation of liver homogenates (post mitochondrial fraction)

Liver (1.0 g) was weighed, cut into bits and thoroughly homogenized in 10 ml of freshly prepared 100 mM phosphate buffer, pH 6.8 to prepare 10% (w/v) homogenates. The homogenates were centrifuged at 3,000 rpm for 10 min using table centrifuge (as earlier described). The supernatants were carefully decanted into clean tubes, labelled, stored frozen and used for protein determination and other biochemical analyses.

Biochemical analyses

i. Plasma and liver alanine and aspartate aminotransferases activities were estimated using Randox Diagnostic kit, according to the procedure of Reitman and Frankel (1957). The activities of the enzyme was extrapolated from the ALT and AST standard calibration curve, respectively and expressed as U/L or U/g of liver.

ii. Gamma Glutamyl Transferase (GGT) activity (Teitz, 1987). The enzyme activity was calculated using the expression:

\[ \text{GGT activity (U/L)} = 1158 \times \Delta A 405 \text{ nm/min; where 1158 (extinction coefficient) and } \Delta A 405 \text{ nm/min (change in absorbance per minute).} \]

iii. The activities of AlkPase and AciPase were assayed according to the procedures of Saini and Van-Ennen (1979) and Oyedapo (1996). The activities were estimated as:

\[ \text{AlkPase activity (U/L)} = 2760 \times \Delta A 405 \text{ nm/min; where 1260 (extinction coefficient) and } \Delta A 405 \text{ nm/min (change in absorbance per minute).} \]

The phosphatase activity was calculated as:

\[ \text{Activity (} \mu \text{mol p-nitrophenol/ml)} = \frac{\text{Abs (410 nm)} \times \frac{1}{\varepsilon} \times 10^6 \times TV}{SV} \]

Where Abs (absorbance at 410 nm), t (period of assay), ε (extinction coefficient, 18.8 × 10² M⁻¹ cm⁻¹), t (1 cm) path length of cuvette, SV (sample volume) and TV (total assay volume), respectively.

iv. The total cholesterol concentration in the plasma was estimated according to the procedure of (Richmond, 1973), HDL-c (Tietz, 1987). Cholesterol concentration of the plasma was calculated using the expression:
RESULTS AND DISCUSSION

The phytochemical screening of the fractions (chloroform [CF], ethyl acetate [EAF], and aqueous [AqF]) and methanolic extract revealed the presence of flavonoids, saponins, tannins, anthraquinone and cardiac glycosides but tannins were not detected in the ethyl acetate fraction (Table 1). Moreover, the levels of total phenolic and flavonoid in the ethyl acetate fraction were 33.58 ± 0.11 mg TAE/g and 50.98 ± 0.89 mg RE/g. This agreed with earlier reports of Wojdylo et al. (2007), who investigated 32 plants for antioxidant activity and phenolic contents. It was noted that 9 of the 32 plants had a higher flavonoid contents than total phenolics. Earlier studies have revealed that flavonoids and phenolics exhibit potent and appreciable biological activities (Olaleye and Rocha, 2008; Lebda et al., 2013).

Due to their simple, accurate reproducibility, paracetamol
and carbon tetrachloride are commonly used to induce hepatotoxicity in animal models (Olatunji, 1999; Colle et al., 2012). Evaluation and assays of hepatic bio-marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase are largely used in the assessment of liver damage by the agents bilirubin and protein (plasma) (Ujah et al., 2014). Membrane damage or necrosis releases the enzyme into the blood and other fluids which are then measured. The elevated levels of these enzymes and metabolites are indication of cellular leakage and loss of functional integrity of the hepatocytes (Rajkapoor et al., 2008; Parmar et al., 2010). In this study, the activity of plasma ALT decreased (4.35%) as a result of the administration of acetaminophen but there was an increase of 10.11% in the liver ALT activity (Table 2). There was a slight increase in the activity of plasma ALT of the treated groups when compared to the acetaminophen-induced group with concomitant decrease in the liver ALT activity. Also, plasma and liver AST activities decreased as a result of the administration of acetaminophen by 9.0% and 26.0%, respectively. However, administration of FF reduced the activities of plasma AST in both pre- and post-treated groups when compared to the acetaminophen-induced group which could be due to the protective effect of FF. The administration of FF slightly reduced liver AST activity of the pre-treated group and slightly increased that of the post-treated group when compared to the acetaminophen-induced group. It could be suggested that FF exhibits both anti-oxidant and pro-oxidant depending on the organ and the response of animals and mode of treatment.

Gamma glutamyl transferase (GGT) and alkaline phosphatase (AlkPase) are membrane bound enzymes, which are released unequally depending on the pathological phenomenon. The elevation of plasma GGT concentrations is regarded as one of the most sensitive indices of hepatic damage (Muthulingam, 2012). The administration of acetaminophen caused increase in the activity of GGT by 82.7% (Table 3) due to leakage of the enzyme from the membrane to the plasma as a result of damage to the membrane which was reduced by 12.74% by the administration of FF. Also elevation of the activity of AlkPase was attributed to the damage of the structural integrity of liver because it is cytoplasmic enzyme and it is released into circulation indicating development of hepatotoxicity (Parmar et al., 2010). The activity of the enzyme was lowered by FF both in pre- and post-treated groups when compared to the acetaminophen induced group. Similar trends were observed in the activities of plasma and liver acid phosphatases in both pre and post treatment groups.

Liver injury of different aetiology is often accompanied by disorders of lipid metabolism in the liver and is often reflected in altered plasma lipids and lipoproteins (Steinberg, 1989; Skottova and Krecman, 1998). In the present study, administration of acetaminophen led to decrease in plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDLC), very low-density lipoprotein cholesterol (VLDLC) but increase in low-density lipoprotein cholesterol (LDLC) levels (Table 4). This was in agreement with earlier observations of Lebda et al. (2013) and Kobashigiana and Kasiska (1997) that oral administration of acetaminophen brought about a decrease in serum triglyceride, HDL-c and VLDL-c levels but an increase in serum LDL-c level with a non-significance difference in the total cholesterol level.

The increase in the TC and LDLC levels in the pre- and post-treated groups could be due to the synergic effects of acetaminophen and FF. The increase in TC of plasma may also be due to the increase in the concentration of acetyl CoA arising from probably enhanced β-oxidation of fatty acid. High blood cholesterol is one of the major risk factors of cardiovascular disease (CVD) (Afolayan et al., 2009). HDLC is considered to have anti-atherogenic properties; therefore increase in HDLC could be clinically beneficial to the animals. The increase in plasma LDLC is understandable since an increase in plasma total cholesterol should normally result in increase in plasma LDL-c (Yakubu et al., 2008). Atherogenic index is referred to as the ratio of LDL-c to HDL-c which has been used as indicator of cardiovascular disease and the cut-offs for high risk of atherosclerosis was put at atherogenic index of greater than 5 (Yakubu et al., 2008). In this study the atherogenic index value was less than 5, it could be therefore inferred that the animals were not likely prone to atherosclerosis. Hence, the use of the extract of P. angolense may be a useful therapeutic agent with treatment and management of CVD.

Cells which are under constant threat of toxic effect of reactive oxygen species (ROS) have a way of eliminating these toxic oxygen species. One major mechanism of this elimination is the antioxidant enzymes cascade (Karan et al., 1999; Radhika et al., 2012). Super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>CF</th>
<th>EAF</th>
<th>AqF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Where (+) represented present and (-) represented absent. EA (ethyl acetate fraction) and AqF (aqueous fraction).

Table 1. Phytochemical Constituents of the Fractions of Methanolic Extraction of P. angolense
Table 2. Plasma and liver ALT and AST activities.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (U/L)</td>
<td>Liver (U/g liver)</td>
</tr>
<tr>
<td>I. Distilled water (control)</td>
<td>79.72±1.52</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>II. Acetaminophen (150 mg/kg bwt)</td>
<td>76.25±3.01*(4.35% ↓)</td>
<td>0.98±0.01*(10.11% ↓)</td>
</tr>
<tr>
<td>III. Silymarin (50 mg/kg bwt)</td>
<td>78.21±2.27</td>
<td>1.02±0.03</td>
</tr>
<tr>
<td>IV. Acetaminophen (150 mg/kg bwt) + Silymarin (50 mg/kg bwt)</td>
<td>77.30±3.29*(1.38% ↑)</td>
<td>1.03±0.01*(6.10% ↑)</td>
</tr>
<tr>
<td>V. FF (125 mg/kg bwt) + Acetaminophen (150 mg/kg bwt) pre-treatment</td>
<td>91.49±6.59**(20.09% ↑)</td>
<td>0.97±0.02**(1.02% ↓)</td>
</tr>
<tr>
<td>VI. Acetaminophen (150 mg/kg bwt) + FF (125 mg/kg bwt) post-treatment</td>
<td>84.70±2.93**(1.18% ↓)</td>
<td>0.98±0.02**(0.0% ↓)</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5. ↑ = increase, ↓ = decrease; a vs. control and b vs. acetaminophen. *p < 0.05 compared to control animals; **p < 0.05 compared to group II; *** not significantly different (group I vs. II); **** not significantly different (group II vs. group V to VI).

Table 3. Plasma GGT, AlkPase and AciPase plasma and liver activities.

<table>
<thead>
<tr>
<th>Group</th>
<th>GGT</th>
<th>AlkPase</th>
<th>AciPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (U/L)</td>
<td>Plasma (U/L)</td>
<td>Plasma (µmol/min/mg protein)</td>
</tr>
<tr>
<td>I. Distilled water (control)</td>
<td>1.16±0.33</td>
<td>9.66±0.80</td>
<td>2.69±0.97</td>
</tr>
<tr>
<td>II. Acetaminophen (150 mg/kg bwt)</td>
<td>2.12±0.39*(82.76% ↑)</td>
<td>11.04±0.0* (14.29% ↑)</td>
<td>3.64±0.53* (35.32% ↑)</td>
</tr>
<tr>
<td>III. Silymarin (50 mg/kg bwt)</td>
<td>1.16±0.41</td>
<td>7.82±0.46</td>
<td>2.80±0.30</td>
</tr>
<tr>
<td>IV. Acetaminophen (150 mg/kg bwt) + Silymarin (50 mg/kg bwt)</td>
<td>1.74±0.0(17.92% ↓)</td>
<td>10.58±1.22 (4.17% ↓)</td>
<td>2.55±0.43 (29.95% ↓)</td>
</tr>
<tr>
<td>V. FF (125 mg/kg bwt) + Acetaminophen (150 mg/kg bwt) pre-treatment</td>
<td>2.17±0.36** (2.36% ↑)</td>
<td>7.94±0.35** (28.08% ↓)</td>
<td>3.39±0.90** (6.87% ↓)</td>
</tr>
<tr>
<td>VI. Acetaminophen (150 mg/kg bwt) + FF (125 mg/kg bwt) post-treatment</td>
<td>1.85±0.22**(12.74% ↓)</td>
<td>10.70±1.04** (3.08% ↓)</td>
<td>2.75±0.48** (24.45% ↓)</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5. ↑ = increase, ↓ = decrease; a vs. control and b vs. acetaminophen. *p < 0.05 compared to control animals; **p < 0.05 compared to group II; *** not significantly different (group I vs. II); **** not significantly different (group II vs. group V to VI).

serve to eliminate primary products of partially reduced oxygen. SODs are found mainly in the intracellular compartment and exist in different forms in the mitochondria matrix, cytosol, and cytoplasm, to lesser extent in the lysosomes and at low concentrations in the extracellular fluid (Geller and Winge, 1984; Halliwell and Gutteridge, 1990).

SODs are involved in the dismutation of the superoxide anion which is an immediate oxygen metabolite with a high biological activity to yield hydrogen peroxide and oxygen (Kharpati et al., 2007). Catalase is present in subcellular peroxisomes and catalyzes the decomposition of hydrogen peroxide to yield oxygen and water (Olaleye and Rocha, 2008; Rapkan et al., 2008). Glutathione peroxidase (GPx) on the other hand catalyses the decomposition of hydrogen peroxide or organic peroxides and reduces glutathione which forms oxidized glutathione (GSSG). GSSG is again reduced to GSH by GPx thus forming redox cycle. These enzymes are found in both cytosol and mitochondria (Afolabi et al., 2012).

In Table 5 is the summary of the effects of the
Table 4. Plasma lipid profile concentration.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>LDL-c/HDL-c ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Distilled water (control)</td>
<td>58.97±2.74</td>
<td>34.40±6.09</td>
<td>44.26±4.77</td>
<td>6.88±1.22</td>
<td>7.83±0.81</td>
<td>0.18</td>
</tr>
<tr>
<td>II. Acetaminophen (150 mg/kg bwt)</td>
<td>33.45±2.33**</td>
<td>23.45±2.33*</td>
<td>18.71±2.53*</td>
<td>4.69±0.78*</td>
<td>10.05±2.26*</td>
<td>0.54</td>
</tr>
<tr>
<td>III. Silymarin (50 mg/kg bwt)</td>
<td>41.28±4.96</td>
<td>23.46±3.90*</td>
<td>18.71±2.53*</td>
<td>4.69±0.78*</td>
<td>10.05±2.26*</td>
<td>0.54</td>
</tr>
<tr>
<td>IV. Acetaminophen (150 mg/kg bwt) + Silymarin (50 mg/kg bwt)</td>
<td>69.73±2.94 (108.46% ↑)</td>
<td>45.24±8.62* (92.84% ↑)</td>
<td>30.12±9.41* (60.98% ↑)</td>
<td>9.05±1.73* (28.35% ↑)</td>
<td>30.56±6.70* (204.08% ↑)</td>
<td>1.01</td>
</tr>
<tr>
<td>V. FF (125 mg/kg bwt) + Acetaminophen (150 mg/kg bwt) pre-treatment</td>
<td>67.47±6.70* (101.70% ↑)</td>
<td>76.55±1.84* (74.16% ↓)</td>
<td>30.12±9.41* (60.98% ↑)</td>
<td>9.05±1.73* (28.35% ↑)</td>
<td>30.56±6.70* (204.08% ↑)</td>
<td>1.01</td>
</tr>
<tr>
<td>VI. Acetaminophen (150 mg/kg bwt) + FF (125 mg/kg bwt) post-treatment</td>
<td>70.09±4.11* (109.54% ↑)</td>
<td>73.38±3.18* (212.79% ↑)</td>
<td>30.64±4.72* (63.76% ↑)</td>
<td>14.68±0.64* (92.96% ↑)</td>
<td>24.78±4.83* (146.57% ↑)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5. ↑ = increase, ↓ = decrease; a vs. control and b vs. acetaminophen. ##p < 0.05 compared to control animals; #p < 0.05 compared to group II; * not significantly different (group I vs. II); ** not significantly different (group II vs. group V to VI).

Table 5. Liver catalase, peroxidase and sod activities.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase</th>
<th>Peroxidase</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver (µmol/min./mg protein)</td>
<td>Liver (U/min./mg protein)</td>
<td>Liver (U/min./mg protein)</td>
</tr>
<tr>
<td>I. Distilled water 5 mg/ml (control)</td>
<td>3.67±0.90</td>
<td>45.40±4.99</td>
<td>59.27±3.20</td>
</tr>
<tr>
<td>II. Acetaminophen (150 mg/kg bwt)</td>
<td>1.58±0.06* (56.95% ↓) b</td>
<td>11.73±1.55* (74.16% ↓) b</td>
<td>9.84±0.52* (83.40% ↓) b</td>
</tr>
<tr>
<td>III. Silymarin (50 mg/kg bwt)</td>
<td>1.53±0.07</td>
<td>11.84±1.95</td>
<td>10.74±0.91</td>
</tr>
<tr>
<td>IV. Acetaminophen (150 mg/kg bwt) + Silymarin (50 mg/kg bwt)</td>
<td>1.39±0.09 (12.03% ↓) b</td>
<td>13.51±3.42 (15.17% ↑) b</td>
<td>9.52±0.65 (3.25% ↓) b</td>
</tr>
<tr>
<td>V. FF (125 mg/kg bwt) + Acetaminophen (150 mg/kg bwt) pre-treatment</td>
<td>2.09±0.12** (32.28% ↑) b</td>
<td>23.98±2.97** (104.43% ↑) b</td>
<td>15.71±5.22** (95.65% ↑) b</td>
</tr>
<tr>
<td>VI. Acetaminophen (150 mg/kg bwt) + FF (125 mg/kg bwt) post-treatment</td>
<td>1.61±0.20** (19.00% ↑) b</td>
<td>15.65±1.77** (33.42% ↑) b</td>
<td>11.28±0.52** (14.63% ↑) b</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5. ↑ = increase, ↓ = decrease; a vs. control and b vs. acetaminophen. ##p < 0.05 compared to control animals; *p < 0.05 compared to group II; ** not significantly different (group I vs. II); *** not significantly different (group II vs. group V to VI).

Acetaminophen, silymarin and flavonoid fraction on the antioxidant enzymes. It was observed that administration of acetaminophen caused drastic reduction in the activities of the enzymes which implied overwhelming of the antioxidant systems by generated ROS. However, treatments (pre and post) led to increase in the activities of the enzymes. The hepatocytes synthesize most proteins found in the plasma and secretes them into circulation. The reduction in plasma and hepatic tissues total protein level could be a result of possible damage to the hepatocytes induced by ingested toxins damage (Radhika et al., 2012). The induction of liver damage with acetaminophen decreased the plasma total protein concentration
Table 6. Total protein (plasma and liver), albumin and bilirubin plasma concentrations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Protein</th>
<th>Albumin</th>
<th>Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (g/dl)</td>
<td>Liver (mg/g)</td>
<td>Plasma (g/dl)</td>
</tr>
<tr>
<td>I. Distilled water 5 mg/ml (control)</td>
<td>3.71±0.50</td>
<td>17.48±1.22</td>
<td>1.78±0.48</td>
</tr>
<tr>
<td>II. Acetaminophen (150 mg/kg bwt)</td>
<td>2.15±0.16* (42.05% ↓)</td>
<td>33.94±5.01* (94.16% ↑)</td>
<td>1.60±0.10* (10.11% ↓)</td>
</tr>
<tr>
<td>III. Silymarin (50 mg/kg bwt)</td>
<td>4.65±0.17</td>
<td>44.71±1.69</td>
<td>2.52±0.24</td>
</tr>
<tr>
<td>IV. Acetaminophen (150 mg/kg bwt) + Silymarin (50 mg/kg bwt)</td>
<td>3.2±0.45 (54.42% ↓)</td>
<td>47.96±3.25 (94.16% ↑)</td>
<td>1.60±0.10 (10.11% ↓)</td>
</tr>
<tr>
<td>V. FF (125 mg/kg bwt) + Acetaminophen (150 mg/kg bwt) pre-treatment</td>
<td>3.74±0.17 (122.79% ↑)</td>
<td>42.88±4.64 (26.34% ↑)</td>
<td>1.90±0.21 (18.75% ↑)</td>
</tr>
<tr>
<td>VI. Acetaminophen (150 mg/kg bwt) + FF (125 mg/kg bwt) post-treatment</td>
<td>4.79±0.13 (122.79% ↑)</td>
<td>42.88±4.64 (26.34% ↑)</td>
<td>1.90±0.21 (18.75% ↑)</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5. *↑ = increase, ↓ = decrease; a vs. control and b vs. acetaminophen. **p < 0.05 compared to control animals; *p < 0.05 compared to group II; ***not significantly different (group I vs. II); ****not significantly different (group II vs. group V to VI).

Table 7. Liver lipid peroxidation and glutathione concentrations.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver (µmol)</td>
<td>Liver (µg/ml)</td>
</tr>
<tr>
<td>I. Distilled water 5 (control)</td>
<td>1.42±0.02</td>
<td>7.53±0.58</td>
</tr>
<tr>
<td>II. Acetaminophen (150 mg/kg bwt)</td>
<td>1.75±0.12* (23.24% ↑)</td>
<td>5.14±0.58* (31.74% ↓)</td>
</tr>
<tr>
<td>III. Silymarin (50 mg/kg bwt)</td>
<td>1.0±0.15</td>
<td>6.63±0.79</td>
</tr>
<tr>
<td>IV. Acetaminophen (150 mg/kg bwt) + Silymarin (50 mg/kg bwt)</td>
<td>0.89±0.30 (49.14% ↓)</td>
<td>7.95±0.59 (54.67% ↑)</td>
</tr>
<tr>
<td>V. FF (125 mg/kg bwt) + Acetaminophen (150 mg/kg bwt) pre-treatment</td>
<td>0.39±0.14* (77.71% ↓)</td>
<td>6.54±0.69* (27.24% ↑)</td>
</tr>
<tr>
<td>VI. Acetaminophen (150 mg/kg bwt) + FF (125 mg/kg bwt) post-treatment</td>
<td>0.88±0.17* (49.71% ↓)</td>
<td>6.37±0.82* (23.93% ↑)</td>
</tr>
</tbody>
</table>

Each value represented Mean ± SEM of n = 5. *↑ = increase, ↓ = decrease; a vs. control and b vs. acetaminophen. **p < 0.05 compared to control animals; *p < 0.05 compared to group II; ***not significantly different (group I vs. II); ****not significantly different (group II vs. group V to VI).

but increased the liver total protein concentration (Table 6). Administration of FF increased the concentration of total protein in the plasma and the liver in the pre- and post-treated groups.

Albumin is the most abundant plasma protein synthesised in the liver, maintains water balance in the plasma and serum, transports and stores a wide variety of ligands (Quinlan et al., 2005). The administration of acetaminophen slightly lowered the concentration of albumin (Table 6) and the concentration was slightly increased in the pre- and post-treated groups.

Total bilirubin a by-product of the breakdown of red blood cells in the liver, is a good indicator of liver function. High levels will cause icterus (jaundice) and are indicative of damage to the liver and bile ducts (Saleem et al., 2008). There was 100% increase in the concentration of bilirubin as a result of acetaminophen administration (Table 6). It was noted that the drug, silymarin and flavonoid fraction altered the concentrations of bilirubin which implied that the compounds might be eliciting adverse effects on the haemoglobin content of the blood and metabolic functions of the kidney. Malondialdehyde (MDA) is one of the end-products of polyunsaturated fatty acid peroxidation and is a good indicator of the degree of lipid peroxidation (Fakurazi et al., 2012; Lodi et al., 2012). The amount of MDA formed increased by 23.24% with the administration of acetaminophen as a result of lipid peroxidation (Table 7). Administration of FF
was able to reduce the amount of MDA formed in both the pre- and the post-treated groups which could be due to ability of the FF to mop up free radicals that exert lipid peroxidation. The results agreed with the earlier observations of Ratty and Das (1988) that flavonoids and phenolic compounds inhibit and reduced the formation of MDA and lipid peroxidation.

Glutathione (GSH) is a tripeptide which plays prominent role in metabolism of both endo and xenobiotics and functions to remove potentially toxic electrophilic compounds and protects the organ against dysfunction (Radhika et al., 2012). The depletion of GSH concentration (Table 7) could not be unconnected with generation of endogenous ROS that are involved in the initiation of lipid peroxidation, membrane breakdown and cell death (Lebda et al., 2013). Administration of FF led to increase in the concentration of GSH in both pre- and post-treated groups which could be as a result of synergetic action of both GSH and FF to mop up excess ROS generated by the acetaminophen. The FF might also be acting by stimulating the activity of glutathione peroxidase to regenerate GSH from GSSG a potent non-enzymatic antioxidant. Flavonoids and phenolics compound impact their biological effects as antioxidants
and free radical scavengers (Afolabi et al., 2012).

Conclusion

It is surmised that flavonoid fraction (FF) of P. angolense exerts its mode of action by eliciting moderate, potent anti-oxidant, free-radical activity and hepatoprotective potential.

Conflict of interest

The authors have not declared any conflict of interest.

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