The Hepatoprotective Effect of *Senna occidentalis* Methanol Leaf Extract Against Acetaminophen-induced Hepatic Damage in Rats

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ABSTRACT

The present study evaluated the hepatoprotective effects and anti-oxidant activities of methanol leaf extract of *Senna occidentalis* against acetaminophen-induced hepatic injury in rats. The plant was extracted by cold maceration in 80% methanol for 48 hours. Acute toxicity test was done in rats orally. Hepatoprotective activity of the extract was investigated in rats challenged with acetaminophen (2000 mg kg⁻¹). Twelve hours post challenge, extract at 75, 150 and 300 mg kg⁻¹ (per os) were administered to rats for 4 days. Silymarin was used as positive control. Serum (alanine aminotransaminase) ALT, (aspartate aminotransferase) AST, (alkaline phosphatase) ALP, total bilirubin and total protein levels were assayed. Pentobarbitone-induced sleeping time was carried out on day 4. The effect of the extract on erythrocyte membrane stability was determined. Concentrations ranging from 10-400 µg mL⁻¹ were assayed (*in vitro*) for antioxidant activities using the DPPH and FRAP spectrophotometric methods. The extract at 150 and 300 mg kg⁻¹ doses significantly (*p*<0.05) reduced acetaminophen-mediated increase in serum transaminases (AST, ALT and ALP) and bilirubin with increase in total serum protein values. The extract exerted maximal effect at 300 mg kg⁻¹. Acetaminophen-induced prolonged pentobarbitone sleeping time was significantly (*p*<0.05) reduced in the groups treated with 150 and 300 mg kg⁻¹ of the extract. Using the DPPH assay, the extract showed 60% antioxidant activity when compared with ascorbic acid (75%) at 400 µg mL⁻¹. FRAP assay gave 1.7 FRAP value compared to 2.0 from ascorbic acid at 400 µg mL⁻¹. The extract markedly stabilized rat erythrocyte cell membranes. The findings suggest that the methanol leaf extract of *S. occidentalis* may be useful in the protection of the hepatocytes from toxins.

Key words: Xenobiotics, hepatotoxicity, *Senna occidentalis*, antioxidant, silymarin, acetaminophen, osmotic fragility

INTRODUCTION

Liver is the largest internal organ of the human body which is part of the digestive system (Karim *et al.*, 2011). It is involved in detoxification of drugs and food substances, deamination of excess proteins, storage of iron, vitamins and glycogen, production of bile, proteins and vital
enzymes in the body. Its pivotal role in biotransformation makes it susceptible to toxic assault by xenobiotics (Craig and Stitzel, 1994). Liver is sometimes damaged due to effects from medications e.g. acetaminophen (Bartlett, 2004), alcohol abuse (Bykov et al., 2004), hepatotoxins (Appiah et al., 2009), autoimmune hepatitis viral and microbial infections (Ardanaz and Pagano, 2006). The roles of Reactive Oxygen Species (ROS) in diverse pathologies stimulate much interest for more effective anti-oxidant agents. Free radicals react with sulphhydryl groups such as glutathione and protein thiol which eventually lead to membrane lipid peroxidation and necrosis (Brauthbar and Williams, 2002). The damaged or dead tissue usually results in the leakage of the enzymes in the affected tissue(s) into the blood stream (Obi et al., 2001). It is however, noteworthy that a large section of the world’s population relies on herbal remedies to treat plethora of diseases due to their low costs, easy access and reduced side effects (Marino-Betlolo, 1980) but the pharmacological basis behind most herbal therapies remains widely undefined.

Some plants with reported hepatoprotective properties are *Garcinia kola* Ker Gaul (Clusiaceae), *Tinospora cordifolia* (A. Rich.), *Ricinus communis* Linn (Euphorbiaceae), *Curcuma longa* Linn (Zingiberaceae), *Enicostemma littorale* Blume (Gentianaceae), *Flaveria trinervia* Linn (Asteraceae) and *Boerhaavia diffusa* Linn (Nyctaginaceae) (Devaki et al., 2004; Umadevi et al., 2004; Vishwakarma and Goyal, 2004). Plants are a major source of novel anti-oxidant and hepatoprotective agents since many industrial drugs are derived as a result of knowledge got from folklore medicine (Brander et al., 1991). Some of the herbal preparations speed up the natural healing processes of the liver (Senthilkumar et al., 2005). *Phellinus rimosus* (Berk) Platt (Hymenochaetaceae), a mushroom has been shown to protect the liver from acute and chronic carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats by restoring the liver anti-oxidant status, inhibiting the phase I and enhancing the phase II enzyme activities (Ajith et al., 2006).

*Senna occidentalis* (L.) Link (Fabaceae) is synonymous with *Cassia occidentalis* L. or *Ditremexa occidentalis* (L), commonly called coffee senna (Haselwood and Motter, 1966). The plant is locally known as stinking weed (Henty et al., 1975). Coffee senna has a single purplish stem and sparse branching. The crushed foliage has an unpleasant odour (Long and Lakela, 1976). Extracts from the plant leaves were repeatedly used folklorically as an analgesic, antibacterial, antifungal, anti-inflammatory, anti-septic, antispasmodic, antiparasitic, antiviral, carminative, diaphoretic, emenagogue, febrifuge, insecticidal, immunostimulant, laxative, purgative, sudorific and vermifuge (Gaind et al., 1966). Ingestion of large amounts of coffee senna seeds caused deaths of cows, goats, horses and pigs (Timm and Riet-Correa, 1997).

The present study was undertaken to evaluate the anti-oxidant activities and hepatoprotective effects of the methanol leaf extract of *Senna occidentalis* against acetaminophen-induced hepatic injury in rats.

**MATERIALS AND METHODS**

**Chemicals, reagents and drugs:** Methanol (Riedel-de Haën AG-hanover), Sodium chloride (BDH, England), Silymarin (Legalon® 70, Chemical Industries, Madaus AG, Germany), 1,1-diphenyl-2-picyrylhydrazyl radical (DPPH) and ferric tripyridyltriazine (Fe (III)-TPTZ) from Sigma Aldrich, Germany, pentobarbitone sodium (Abbot Laboratories Ltd. Kent, UK), ascorbic acid and acetaminophen from Sigma, Germany were used for the study. Kits for serum alanine aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) were purchased from Randox Laboratories Ltd. United Kingdom, total bilirubin and total protein laboratory kits were purchased from Quimica Clinica Applicada S.A., Spain. The study was carried out in October, 2010.
**Animals:** Adult wistar albino rats (130-180 g) obtained from the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. Animals were kept in stainless steel cages and had access to feed (Vital feed8, Nigeria Ltd.) and water *ad libitum* except in situations where fasting was required. The rats were allowed 14 days to acclimatize before the experiments were conducted according to the permission and prescribed guidelines of the Institutional Animal Ethics Committee. A total number of 70 albino rats were used for the experiments.

**Plant materials and extraction:** Fresh leaves of *S. occidentalis* were collected from Obollo Afor in Udenu local government area, Enugu State, Nigeria, identified and a voucher sample was kept in the departmental herbarium (UNN/BD.4123.09), Department of Botany, University of Nigeria, Nsukka. The leaves of *S. occidentalis* were dried at room temperature and pulverized into coarse powder using hammer mill. One kilogram of the powdered leaves was extracted by cold maceration with 80% methanol and intermittent vigorous shaking for 48 h. The extract was filtered and concentrated in *vacuo* to dryness with a rotary evaporator. The concentration and percentage yield of the extract were determined.

**Acute toxicity test:** Thirty matured albino Wistar rats of both sexes were weighed and randomly separated into 6 groups (A-F) of 5 rats per group. Groups A-E were dosed orally with varying doses (150, 300, 600 and 1500 mg kg\(^{-1}\)) of the leaf extract of *S. occidentalis* plant, respectively while group F (6 group) was given an equivalent volume of distilled water (10 mL kg\(^{-1}\)). The rats were allowed access to feed and water *ad libitum* for 48 h and observed for signs of toxicity and death.

**Determination of osmotic fragility:** The effect of the methanol leaf extract of *S. occidentalis* on erythrocyte membrane stability as determined by mean corpuscular fragility was studied using the method of Parpart *et al.* (1947) as modified by Elekwa *et al.* (2008).

**Effects of *S. occidentalis* leaf extract of acetaminophen-induced hepatotoxicity in rats:** A total of thirty-six (36) male wistar rats were separated into 6 groups comprising of 6 animals in a group. All the rats, except group 1 (normal) were challenged orally with a single dose (2000 mg kg\(^{-1}\)) of acetaminophen. After the 12 h challenge with acetaminophen, group 2 (positive control) received distilled water (10 mL kg\(^{-1}\)) only for 4 days. Group 3 rats were given silymarin (50 mg kg\(^{-1}\), p.o.) for 4 days and this served as the standard for comparison. Groups 4, 5 and 6 acetaminophen-challenged rats were treated with 75, 150 and 300 mg kg\(^{-1}\) of the leaf extract of *S. occidentalis*, respectively for 4 days. Treatments were orally with gastric gavage. Pentobarbitone-induced sleeping time was carried out on day 4 by intraperitoneal administration of pentobarbitone sodium (35 mg kg\(^{-1}\), p.o.). The sleeping time was calculated as the interval between the loss and recovery of the wrighting reflex (Shetty and Anika, 1982).

On recovery from anesthesia, blood samples were collected and the rats humanely sacrificed. Blood samples were collected into test tubes and allowed to clot at room temperature. The blood samples were centrifuged at 2,500 rpm/10 min to separate the serum and were used to determine the serum ALT, AST, ALP, total bilirubin and total protein levels.

**Biochemical assay:** The serum levels of ALT, AST were determined using the method of Reitman and Frankel (1957), serum ALP level by the method of Kind and King (1954), total
bilirubin level by the method of Malloy and Evelyn (1937) as modified by Tietz (1996) and total serum protein level by the method of Johnson (1943).

**Antioxidant activity of the extract of *S. occidentalis* leaves with DPPH photometric Assay:** The free radical scavenging activity of the extract of *S. occidentalis* leaves was evaluated by the DPPH assay following a standard method of Mensor et al. (2001).

**Estimation of the anti-oxidant activity of *S. occidentalis* using Ferric Reducing/Antioxidant Power (FRAP) method:** The test was carried out following a standard procedure of Benzie and Strain (1999).

**Statistical analysis:** All data collected were subjected to one-way analysis of variance (ANOVA) and Duncan’s Multiple Range Test (DMRT). Differences at p<0.05 were considered significant.

**RESULTS**

**Plant extraction and acute toxicity:** The plant extraction yielded 18.74% w/w dry matter and the experimental animals exhibited no sign of toxicity during the test period.

**Osmotic fragility:** The lowest test concentration (0.2 mg mL⁻¹) of *S. occidentalis* extract induced 5.2±1.4 mean corpuscular fragility in rat erythrocyte membranes when compared with the highest concentration (1.0 mg mL⁻¹) of the extract induced 3.8±1.7 osmotic fragility in the erythrocyte membranes (Table 1, Fig. 1).

**Pentobarbitone-induced sleeping time:** The pentobarbitone-induced sleeping time was significantly (p<0.05) prolonged in acetaminophen-challenged, untreated rats (158.7±25.36)

![Graph showing sleeping time](image)

**Fig. 1:** The effect of methanol extract of *S. occidentalis* and Silymarin on sleeping time in rats intoxicated with acetaminophen

<table>
<thead>
<tr>
<th>Concentration of extracts (mg mL⁻¹)</th>
<th>MCF expressed as [NaCl]g L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>5.2±1.4*</td>
</tr>
<tr>
<td>0.6</td>
<td>4.0±2.4</td>
</tr>
<tr>
<td>1.0</td>
<td>3.8±1.7</td>
</tr>
<tr>
<td>Control</td>
<td>3.5±1.4</td>
</tr>
</tbody>
</table>

*Significantly different from control group; p<0.05
Fig. 2: The antioxidant property of *S. occidentalis* crude extract with FRAP assay

Table 2: Effects of the methanol leaf extract of *S. occidentalis* on serum AST, ALT, ALP, Total bilirubin and Total Protein of Acetaminophen-induced hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>AST (µ L⁻¹)</th>
<th>ALT (µ L⁻¹)</th>
<th>ALP (µ L⁻¹)</th>
<th>Total bilirubin (mg dL⁻¹)</th>
<th>Total protein (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control distilled water</td>
<td>53.13±1.0*</td>
<td>75.09±2.85*</td>
<td>25.29±2.90*</td>
<td>0.19±0.004*</td>
<td>7.01±0.15*</td>
</tr>
<tr>
<td></td>
<td>(10 mL kg⁻¹) (No acetaminophen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Acetaminophen (2000 mg kg⁻¹) only</td>
<td>131.49±0.87*</td>
<td>153.22±1.89*</td>
<td>53.94±0.88*</td>
<td>2.11±0.008*</td>
<td>3.99±0.25*</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (50 mg kg⁻¹)</td>
<td>63.87±1.29*</td>
<td>79.95±1.71*</td>
<td>29.81±1.70*</td>
<td>0.15±0.002*</td>
<td>5.99±0.019*</td>
</tr>
<tr>
<td>IV</td>
<td>Senna leaf extract (75 mg kg⁻¹)</td>
<td>127.75±1.60*</td>
<td>138.49±3.01*</td>
<td>38.99±0.96*</td>
<td>1.99±0.18*</td>
<td>5.59±0.15*</td>
</tr>
<tr>
<td>V</td>
<td>Senna leaf extract (150 mg kg⁻¹)</td>
<td>87.90±3.01*</td>
<td>93.78±0.98*</td>
<td>36.14±1.96*</td>
<td>0.99±0.34*</td>
<td>6.01±0.15*</td>
</tr>
<tr>
<td>VI</td>
<td>Senna leaf extract (300 mg kg⁻¹)</td>
<td>59.99±1.98*</td>
<td>77.01±2.00*</td>
<td>31.89±1.70*</td>
<td>0.44±0.08*</td>
<td>5.97±0.20*</td>
</tr>
</tbody>
</table>

*Significantly different from Group I; p<0.05. = Significantly different from normal control group (Group I); p<0.05

compared to the normal rats, Group I (98.12±15.08). Silymarin (50 mg kg⁻¹) gave 109.54±08.21 while the extract gave 101.94±18.47 and 115.71±25.36 at 150 and 300 mg kg⁻¹ respectively at p<0.05 and also decreased prolonged pentobarbitone-induced sleeping time when compared with acetaminophen-challenged, untreated rats (Fig. 2).

**Acetaminophen-induced liver toxicity:** The serum ALT, AST and ALP levels were significantly (p<0.05) increased in the acetaminophen-challenged, untreated group II rats (131.49±0.87, 153.22±1.89, 53.94±0.93), respectively (Fig. 2) when compared to non-challenged normal group (53.13±1.0, 75.09±2.85, 25.29±2.90). The leaf extract of *S. occidentalis* at 150 p.o. significantly (p<0.05) decreased the elevated levels of AST, ALT and ALP as 87.90±3.01, 93.78±0.98 and 36.14±1.90, respectively while 300 mg kg⁻¹ gave 59.99±1.98, 77.01±2.00 and 31.89±1.70 respectively (Table 2). Similarly, silymarin (50 mg kg⁻¹) and *S. occidentalis* extract (150 and 300 mg kg⁻¹) caused a significant (p<0.05) reduction in the total serum bilirubin level but a significant (p<0.05) increase in the total protein level of test rats when compared to acetaminophen-challenged, untreated group.

**Antioxidant activity of *S. occidentalis* extract:** The methanol extract of *S. occidentalis* leaves gave 0.58, 0.48, 1.7 antioxidant power at 100, 200 and 400 µg mL⁻¹ respectively with FRAP assay (Fig. 2). DPPH assay showed 60% antioxidant activity at 400 µg mL⁻¹, 55 at 200 µg mL⁻¹ and 49 at 100 µg mL⁻¹ of the crude extract while ascorbic acid had 79% at the same concentration (Fig. 3).
Fig. 3: The antioxidant activity of the crude extract of *S. occidentalis* leaves and ascorbic acid determined with DPPH radical scavenging method

**DISCUSSION**

Some chemicals including environmental toxicants can induce severe cellular damage in different organs of the body through the metabolic activation of highly reactive oxygen species (Bashandy and AlWasel, 2011). Protection against acetaminophen-induced toxicity has been used as a test for potential hepatoprotective activity by many scientists (Ahmed and Khater, 2001). Hepatic cells contain higher concentrations of AST and ALT in the cytoplasm and AST in particular exists in the mitochondria (Wells, 1988). Damage to hepatic cells induces leakage of plasma to cause an increased level of hepato-specific enzymes in serum (Tolman and Rej, 1999). The measurement of serum AST, ALT and ALP levels serve as a means for indirect assessment of liver function.

Antioxidants are among the first link between chemical reactions and biological activities (Trouillas et al., 2003) and they block the process of oxidation by neutralizing free radicals. The hepatoprotective index of a drug can be evaluated by its capability to reduce the injurious effects induced by a hepatotoxin or to preserve the normal hepatic functions (Kumar et al., 2009). CCl₄⁻ and/or CCl₃OO⁻ radicals produced as a result of metabolic conversion of CCl₄ are reported to initiate lipid peroxidation and cellular damages (Gupta et al., 2006).

The leaf extract of *S. occidentalis* and silymarin reduced the serum levels of AST, ALT and ALP and also preserved the functional ability of the liver. A similar observation was made by Samudram et al. (2008). This was revealed when 150 and 300 mg kg⁻¹ of the extract produced significant (p<0.05) reduction in total bilirubin but increased total protein levels relative to acetaminophen-intoxicated, untreated rats (Table 2). The liver is the site for the production of various physiological proteins. On exposure to the hepatotoxin like acetaminophen, CCl₄ etc., the amount of total protein present in the serum decreases due to less activity of the hepatocytes (Krishna et al., 2009). The conjugating and synthesizing ability of the liver was therefore, intact. The decreased pentobarbitone-induced sleeping time at both doses of the extract (150 and 300 mg kg⁻¹, respectively) also gave credence to the protection given to the liver such that hepatocytes remained viable to carry out biotransformation of the drug (Fig. 1). The extract did not show appreciable hepatoprotective activity at 75 mg kg⁻¹ even though there was decrease in serum ALP level and elevated total protein value when compared with acetaminophen-intoxicated, untreated rats (Table 2).

The extract of *S. occidentalis* leaves demonstrated a high antioxidant potential. In the crude form it gave concentration-dependent increase in antioxidant power and 60% antioxidant activity
compared to 79% with ascorbic acid, a reference antioxidant at the same concentration in the DPPH in vitro assay (Fig. 3). Many plant extracts have indicated that they could in different ways effectively protect liver from active metabolites of acetaminophen and free radicals produced by carbon-tetra chloride (Fakurezi et al., 2008). This is in line with the works of Natanzi et al. (2010). Vitamin C (ascorbic acid) might improve oxidative damage by decreasing lipid peroxidation by altering antioxidant defense system (El-Gendy et al., 2010). A number of investigators have previously demonstrated that antioxidants prevent CCl₄ toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation (Teselkin et al., 2000), suppressing Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities (Lin and Huang, 2000). The extract markedly stabilized rat erythrocyte cell membranes; hence the lowest concentration (0.2 mg L⁻¹) produced increased osmotic fragility (Table 1). Toxic injury to hepatocytes stimulates inflammatory reactions. Most of the hepatotoxic chemicals including acetaminophen damaged liver mainly by inducing lipid peroxidation directly or indirectly (Sadasivan et al., 2006). The cell membrane damage associated with inflammation results in leucocyte release of lysosomal enzymes that can be injurious to nearby cells (Konturek et al., 2000). Cell damage causes the release of arachidonic acid and pro-inflammatory cytokines. Stimulation of neutrophils can lead to the production of oxygen-derived free radicals that produce further cellular damage. The mechanism of the hepatoprotective activity of S. occidentalis extract in acetaminophen intoxicated rats may be derived from some anti-inflammatory and antioxidant principles in the extract and also, stabilization of cell membranes. It was also possible for the extract to cause accelerated regeneration of the liver cells.

CONCLUSION

S. occidentalis extract significantly reduced serum levels of AST, ALT, ALP and total bilirubin but increased total serum protein level which may suggest hepatoprotection. This was further supported by a reduction in pentobarbitone-induced sleeping time and osmotic fragility of cells.

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