Tropical Journal of Natural Product Research

Protective Effect of Ethanol Extract of *Senna occidentalis* Leaf Against Cadmium-Induced Hepatotoxicity in Rats

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ARTICLE INFO

ABSTRACT

Cadmium is a known environmental and industrial toxicant exposure to which leads to the generation of reactive oxygen species (ROS), with attendant oxidative damage to membrane lipids, cellular proteins and DNA. Recently, the use of medicinal plants as prophylactic or therapeutic agents against certain diseases has become popular. This study was aimed at assessing the hepatoprotective effect of ethanol extract of *Senna occidentalis* leaf in cadmium intoxicated rats. Thirty-five rats, each weighing on the average 150 ± 40 g were randomly selected into seven groups of five rats each. Each group received appropriate treatment. At the end of the treatment period, each rat was sacrificed and the antioxidant status determined using extant methods. The data collected were expressed as mean $\pm$ SD and statically analyzed using one-way analysis of variance (ANOVA). The results showed that cadmium intoxication significantly increased liver malondialdehyde (MDA) levels but caused decrease in superoxide dismutase (SOD) and catalase (CAT) activities as well as reduced glutathione levels compared to control. The level of fragmented DNA also increased significantly. However, co-treatment of rats with cadmium chloride and the extract significantly reduced the concentrations MDA and the severity of the DNA fragmentation. Along with these are concomitant increase in SOD and CAT activities and GSH concentration. Ethanol extract of *Senna occidentalis* leaf possesses bioactive agents capable of protecting liver against cadmium-induced lipid peroxidative membrane damage. The hepatoprotection potential of the leaf extract is likely due to the boost in the organs antioxidant capacity.

Introduction

From antiquity, humanity has been bedeviled with various diseases. In recent times, pathological conditions like organ failure, cardiovascular and atherosclerotic problems, cancer, developmental disabilities, Alzheimer's disease and more have been added. This increase is believed to be the consequence of increased exposure to heavy metals. Small exposure to heavy metals and other chemicals (especially at vulnerable developmental stages) can have long term health impacts[1]. Heavy metals can accumulate in the tissues of human, animals and aquatic organisms which trigger the production of a range of hazardous effects in a cell, or entire organ or whole organism[2]. Among the toxic heavy metals present in the environment, cadmium is the most abundant and concern for its potential impact on public health[3] is increasing worldwide.

In recent years, the attention of scientific investigators has been drawn to the "health-promoting" activity of medicinal plants[4]. *Senna occidentalis,* an annual shrub native to tropical and subtropical region of America, but has naturalized in Africa, Asian, Australia and Southern and Eastern USA. It belongs to the family Leguminosae and the subfamily Caesalpiniaceae[5]. There are reports its ethnomedicinal use as remedy for several human and animal ailments[6, 7]. The stem bark and leaf extracts of *S. occidentalis* contain phytochemicals such as anthraquinones, reducing sugar, glycosides, cardiac glycosides, steroids, alkaloids, flavanoids, saponins, tannins, phenols, phytosterols, gum and mucilage[8, 9]. Some bioactive compounds[10] and a number of pharmacological activities of *Cassia occidentalis* have been reported of in Literature[11-17].

C. occidentalis, a species in the same family is known to exert antioxidant effect by quenching free radicals and singlet form of molecular oxygen[18]. *In vitro* study of the ethyl acetate fraction of whole plant of *C. occidentalis* exhibited antioxidant activity on 1, 1-diphenyi-2-picrylhydrazyl (DPPH) radicals in nitric oxide and hydrogen peroxide scavenging assays[19]. Chrysophanol, an important phytochemical in *C. occidentalis*, was reported to decrease lipid peroxidation[14]. Similarly, the ethanol extract of *C. occidentalis* leaf was reportedly able to prevent carbon tetrachloride-induced hepatotoxicity in rats by increasing the level of antioxidant defense enzymes in liver of rats as evidenced by the increased activities of the enzymes in the homogenate[20, 21]. Whole plant of *C. occidentalis* ethanol extract had also been shown to exert significant inhibitory effect on rat liver microsomal peroxidation[16]. This study was designed to assess the hepatoprotective effect of ethanol extract of *Senna occidentalis* leaf in cadmium intoxicated rats.

Materials and Methods

Plant Sample Collection and Preparation of Extracts

Fresh leaves of *Senna occidentalis* (L.) were collected from the premises of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. Plant was identified and authenticated at the Department of Biology, LAUTECH, where a specimen voucher number (LHO478) was issued and deposited in the Herbarium Unit. The leaves were air-dried for four weeks, and then pulverized using a mechanical grinder. The powder was macerated in 2.5 L of 98% ethanol for 72 hours. The mixture was filtered and the filtrate concentrated. The ethanol concentrate was evaporated to dryness in a rotary evaporator. The extract was stored in an airtight container at room temperature and kept until required for the studies.

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Animal Procurement and Treatment

Male *albino* rats (wistar strain), mean weight 150±40g, were obtained from a commercial breeder in Ogbomoso, Oyo State. After

randomization, the rats were acclimatized for a period of 14 days under standard laboratory conditions (25 ± 2°C), relative humidity of 60 ± 5% and 12 hours light/dark cycle. Before and during the experiment, rats were maintained on standard pellets and water given ad libitum.

Experimental Design

Albino rats of the wisbar strain were intoxicated by intra-peritoneal injection of freshly prepare solution of cadmium chloride (1 mg/kg) in normal saline, (0.9% NaCl in H_2O, w/v) every other day for 14 days [22]. The rats were administered the test extract for 14 days. The rats were divided into seven experimental groups of 5 rats each as follows: Group I: Rats in this group received 0.1 ml of normal saline for 14 days. Group II: Rats in this group received 1 mg/kg cadmium chloride in 0.1ml of normal saline for 14 days. Group III: Rats here received cadmium chloride (1 mg/kg) and 100 mg/kg extract each in 0.1ml concomitantly for 14 days. Group IV: Rats in this group received cadmium chloride and 2.5 mg ramilpril/kg each in 0.1ml concomitantly for 14 days. Group V: In this group, rats received cadmium chloride and combination therapy of the extract and ramilpril for 14 days. Group VI: In this group, rats received 100 mg extract/kg for 14 days. Group VII: Rats in this group received 2.5 mg ramilpril/kg for 14 days.

Biochemical Analysis

MDA level was determined according to the method described by Rice-Evans *et al.* [23]. The sample (0.4 mL) was mixed with 1.6 ml of Tris-KCl buffer to which 0.5 ml of 30% TCA was added. TBA (0.5 mL) was then added and the mixture was heated in water bath at 80^0C for 45 minutes. The mixture was then cooled in ice and centrifuged for 15 minutes at 3000 rpm. The resulted pink solution was measured at an absorbance of 532 nm against a reference blank of distilled water and MDA level was calculated. GSH concentration was measured as described by Anderson (1985) [24]. The sample (0.1 mL) was diluted with 0.9 ml of distilled water and 3 ml of 4% sulphosalicyclic acid solution was added. The mixture was centrifuged at 3,000 x g for 10 minutes. The supernatant (0.5 mL) was mixed with 4 mL of 0.1 M phosphate buffer and 4.5 mL of Ellman's Reagent was added. A blank was prepared with reaction mixture of 4 mL of 0.1 M phosphate buffer, 0.5 mL of the diluted precipitating solution and 4.5 mL of Ellman's Reagent. All readings were taken within 5 minutes at 412 nm, as color developed is not stable. All measurements were taken at absorbance of 412 nm. GSH concentration was determined by plotting a standard curve for GSH and the equivalent concentration for absorbance values ere extrapolated from the curve.

SOD activity was determined by the method of Misra and Fridovich [25]. Briefly, 1 mL of the sample was diluted in 9 mL of distilled water. The diluted sample (0.2 mL) was added to 2.5ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5 mL buffer, 0.3ml of substrate (adrenaline) and 0.2 ml of water. The absorbance at 480nm was monitored every 30 seconds for 150 seconds and the SOD activity was calculated.

CAT activity was determined according to the method reported by Sinha (1972) [26]. Briefly, 1 mL of the sample was mixed with 49 mL of distilled water to give a 1 in 50 dilution of the sample. The mixture contained 4 ml of H_2O_2 solution (800 μmoles) and 5 ml of Phosphate buffer in a 10 mL flat bottom flask. A properly diluted enzyme preparation (1 mL) was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. A 1 mL portion of the reaction mixture was blown into 2 ml of dichromate acetic acid reagent at 60s intervals. The remaining hydrogen peroxide is determined by measuring chromic acetate colorimetrically after heating the reaction mixture. The mononuclear velocity constant k for the decomposition of H_2O_2 catalase was determined using the equation for a first-order reaction according to the equation below:

$$k = \frac{\log (S_0/S)}{t}$$

Where S_0 is the initial concentration of the hydrogen peroxide and S is the concentration of the peroxide at time t minute. The values of the k are plotted against time in minutes and the velocity constant of catalase k_0 at 0 minute determined by extrapolation. The catalase constants of the enzyme preparation were expressed in terms of katalase feiahigkeit or "kat f".

$$\text{Kat f} = \frac{Ko}{mg\ protein\ (ml)}$$

$$Ko = H_2O_2\ consumed$$

$$H_2O_2\ remained = \frac{Change\ in\ absorbance/min}{0.171}$$

$$H_2O_2\ consumed = 800 - H_2O_2$$

The diphenylamine (DPA) method described by Wu *et al.* [27] was employed to assess the endonuclease cleavage products of apoptosis. The liver was homogenized in 10 volume of Tris-EDTA (TE) buffer and centrifuged at $27000 \times g$ for 10 minutes, to separate the intact DNA (pellet) from the fragmented DNA (supernatant). Both the pellet and supernatant (5ml each) were treated with freshly prepared DPA reagent (3ml) for colour development. Incubation was carried out at $37\ ^0C$ for 16-24 hours. The absorbance of the light green or yellowish green supernatant was read spectrophotometrically at 620 nm and the % fragmented DNA was calculated.

Statistical Analysis

Data collected were expressed as mean ± SD and subjected to one-way analysis of variance (ANOVA) using the statistical software SPSS 16.0 (SPSS Inc. Chicago, IL, USA). Least significant differences (LSD) were used as a test of significance within treatments. A p-value of ≤ 0.05 was considered statistically significant.

Results and Discussion

The biological production of reactive oxygen species, primarily superoxide anion (O_2-) and hydrogen peroxide (H_2O_2), causes lipid peroxidation [28]. This is evidenced by increased tissue level of malondialdehyde (MDA) and other polyunsaturated fatty acid degradation products [29]. Lipid peroxidation products in mammals generally cause neurotoxicity, hepatotoxicity and nephrotoxicity [30]. Figure 1 shows the level of MDA in the liver of rats from the various treatment groups. The MDA level were significantly increased ($p < 0.05$) in cadmium intoxicated rats when compared to the normal control. This was consistent with the report that cadmium is an inducer of oxidative stress and lipid peroxidation [31] in exposed organisms.

Treatment with ethanol extract of *S. occidentalis* leaf (group III) and combination of the extract and ramilpril (Group V) caused significant decrease ($p < 0.05$) in MDA level when compared to group II rats. The ethanol extract when administered alone to rats (group VI) led to significant reduction ($p < 0.05$) of the MDA level compared to the control. This is consistent with the finding of other investigators [16] that *S. occidentalis* leaf extract impairs lipid peroxidation.
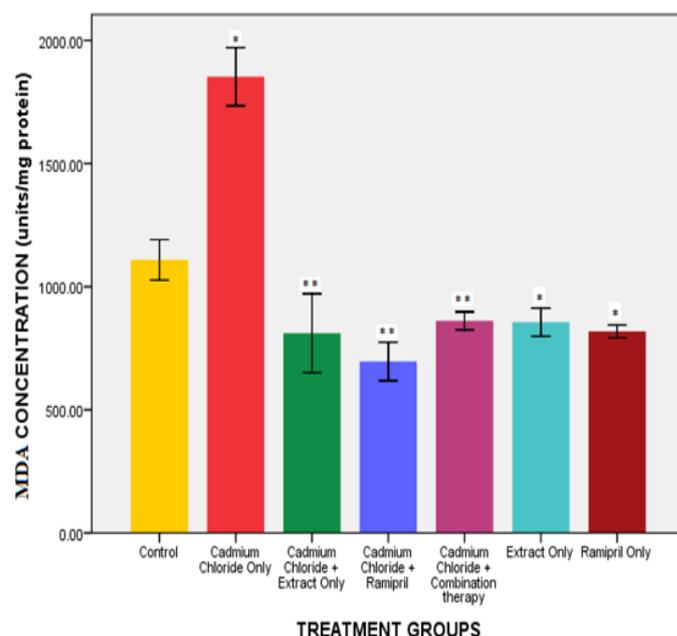

Figure 1: Malondialdehyde (MDA) level in liver.
Values are expressed as means ± SD, n = 5
* Value differs significantly from control (Group I) (P ≤ 0.05).
** Value differs significantly from Cadmium intoxicated group (Group II) (P ≤ 0.05).

In response to reactive oxygen species (ROS) assaults, cells have antioxidant defense system which combats the chain reaction of free radicals. Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It constitutes the first line of defense against free radicals and plays central role in maintaining the cell's redox state [32]. Furthermore, superoxide dismutase (SOD) and catalase (CAT) can scavenge free radicals and ROS such as superoxide radicals and hydrogen peroxide [33, 34].

In this study, results in Figure 2 shows that there was a significant decrease ($p < 0.05$) in the hepatic concentration of GSH in cadmium intoxicated rats when compared to the control. This is in agreement with an earlier report in which the authors [35] suggested that cadmium stimulates the production of and subsequently binds to various biological molecules containing sulfhydryl groups, thereby decreasing their cellular levels. However, there was a significant increase ($p < 0.05$) in liver GSH concentration of rats exposed to the extract (group III) and to the combination treatment (group IV) when compared to the group II rats. Liver SOD (Figure 3) and CAT (Figure 4) activities were significantly decreased ($p < 0.05$) in cadmium intoxicated rats relative to control. Ikediobi and colleagues reported similar trends in the activities of these antioxidant enzymes during cadmium-induced oxidative stress [36].

However, oral administration of the leaf extract to cadmium intoxicated rats (group III) significantly increased ($p < 0.05$) the activities of SOD and CAT when compared to the untreated cadmium intoxicated rats. A similar effect was observed in rats that received the extract and ramilpril besides cadmium. In addition, the administration of the extract alone to cadmium-free rats led to a significant increase ($p < 0.05$) in hepatic SOD and CAT activities in contrast to the control. This is consistent with the findings of Rani and his associates [14] on antioxidant and hepatoprotective activity of *C. occidentalis* in paracetamol-induced rats. Cadmium is a potential carcinogen [37, 38]. Reports indicate that it produces DNA single strand breaks, DNA-protein cross-links, chromosomal aberrations and changes the expression of proto-oncogene and so induces apoptosis [39].
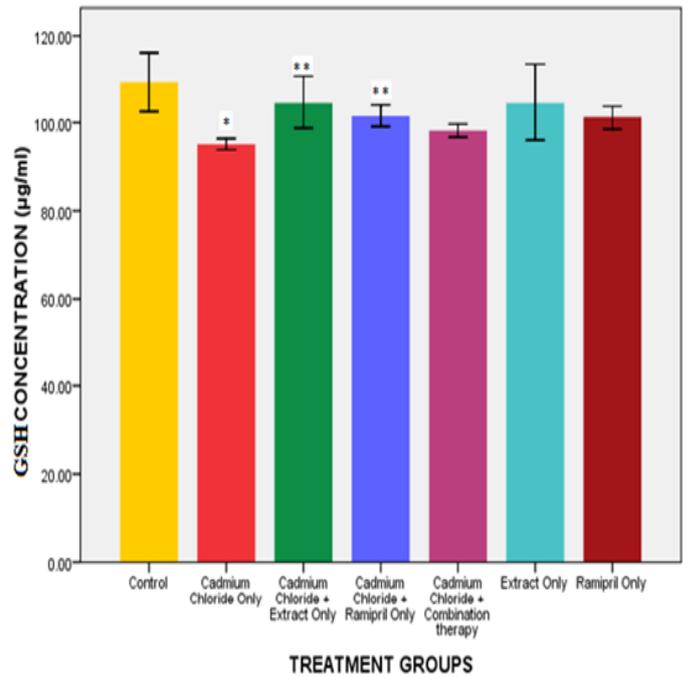

Figure 2: Liver Reduced glutathione (GSH) concentration.
 Values are expressed as means ± SD, n = 5
* Value differs significantly from control (Group I) (P ≤ 0.05).
** Value differs significantly from Cadmium intoxicated group (Group II) (P ≤ 0.05).
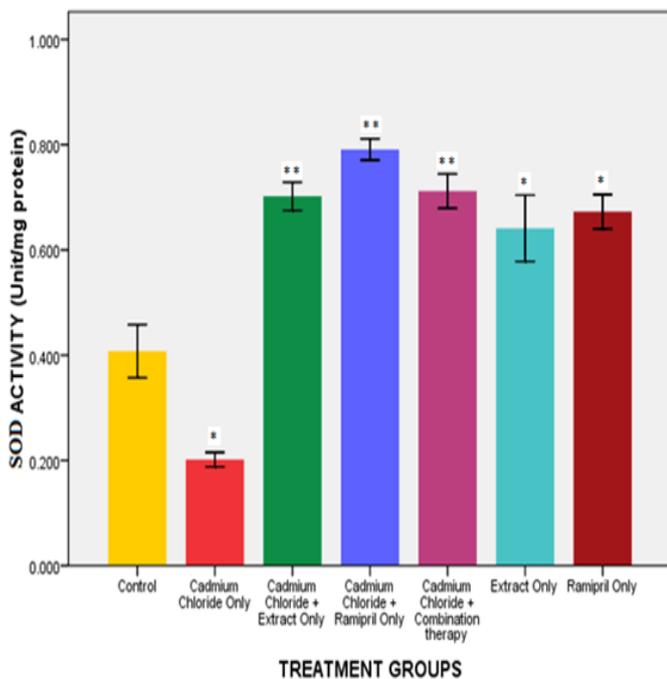

Figure 3: Liver Superoxide dismutase (SOD) activity.
Values are expressed as means ± SD, n = 5
* Value differs significantly from control (Group I) (P ≤ 0.05).
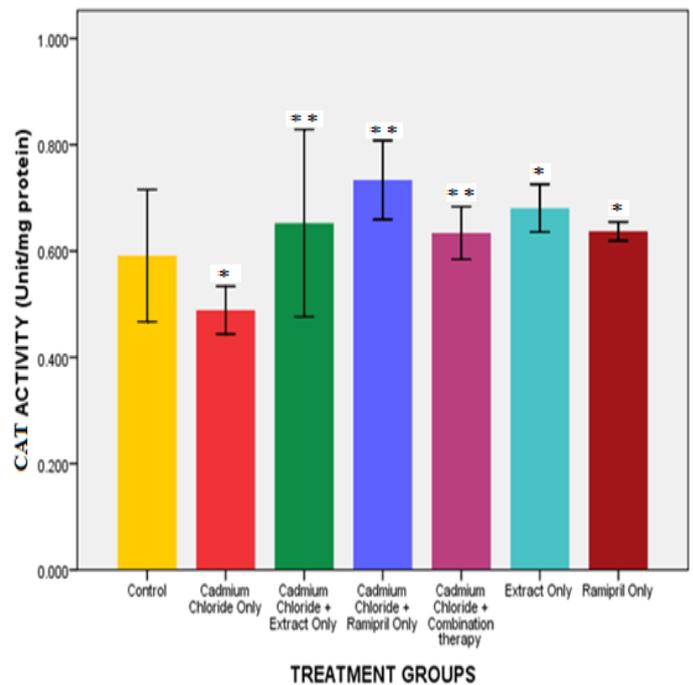** Value differs significantly from Cadmium intoxicated group (Group II) (P ≤ 0.05).

Figure 4: Liver Catalase (CAT) activity.
Values are expressed as means ± SD, n = 5
* Value differs significantly from control (Group I) (P ≤ 0.05).
** Value differs significantly from Cadmium intoxicated group (Group II) (P ≤ 0.05).

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A study suggests that low levels of cadmium inhibit the activities of phosphatases and kinases that are involved in DNA repair [40]. Experiments on animal models and cell lines showed that cadmium is likely involved in the pathogenesis of cancers in organs like breast, lung, prostrate and kidney [41]. Figure 5 shows a significant increase (p<0.05) in the level of fragmented hepatic DNA in cadmium intoxicated rats in contrast to the control. However, administration of the extract and extract combined with ramilpril to the rats significantly decreased (p < 0.05) the levels of fragmented DNA when compared to the untreated rats. Moreover, administration of the extract alone to cadmium-free rats caused a significant decrease (p < 0.05) in the levels of fragmented DNA compared to the group III rats.

However, no significant difference (p>0.05) was observed in cadmium-free rats given the extract relative to the cadmium-free control rats. This is in harmony with the earlier reports that the extract of *C. occidentalis* exhibits anti-mutagenic, anti-carcinogenic [11, 15] and hepatoprotective activities [15].
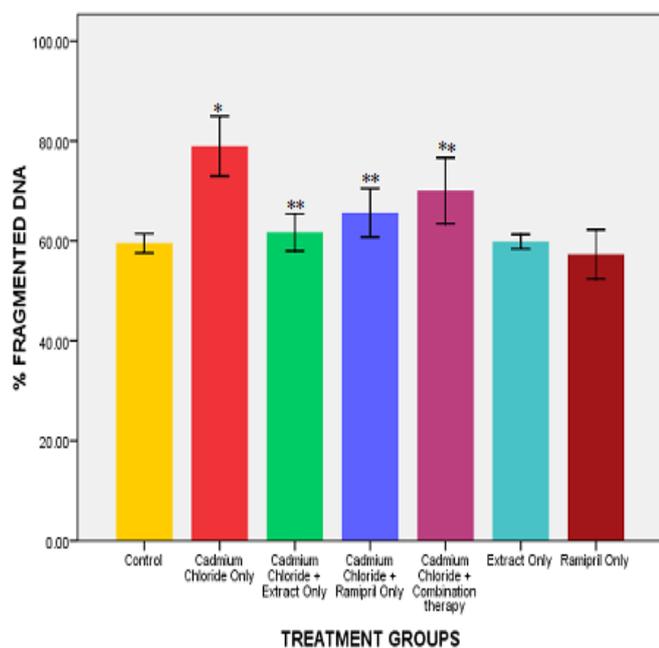

Figure 5: Liver DNA fragmentation.
 Values are expressed as means ± SD, n = 5
* Value differs significantly from control (Group I) (P ≤ 0.05).
** Value differs significantly from Cadmium intoxicated group (Group II) (P ≤ 0.05).

Conclusion

The results of this study indicate that exposure to cadmium induces oxidative damage in the liver by altering the balance between pro-oxidants and antioxidants in favour of pro-oxidants hepatocytes enhancing peroxidation of membrane lipids. Cadmium caused reduction in SOD and CAT enzyme activities, and GSH levels in the liver of rats. It induced pronounced DNA damage in hepatocytes. Administration of ethanol extract of *Senna occidentalis* leaf significantly reversed the cadmium-induced hepatic dysfunctions. Therefore, the mechanism by which the extract protected the liver from cadmium associated injury as is evident in this study is via the enhancement of tissue antioxidant. However, more studies are required for its enzyme activities and increased liver level of glutathione in the reduced state.

Conflict of interest
The authors declare no conflict of interest.

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