In vitro Antioxidant/ Radical Scavenging Activities and Hepatoprotective Roles of Ethanolic Extract of *Cassia occidentalis* Leaves in Sodium Arsenite-Treated Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between the authors. Author MAG designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OAO contributed to the literature searches and corrected the draft manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aims: To investigate *in vitro* antioxidant/radical scavenging activities and hepatoprotective ability of ethanolic leaf extracts of *Cassia occidentalis* (COLEX) in male Wistar rats treated with sodium arsenite ($NaAsO_2$).
Study Design/Methodologies: Using four different methodologies, the anti-oxidant/free radical scavenging activities of COLEX were determined in comparison with standard antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). For the hepatoprotection study, four groups of rats were used. Groups **A**: Control group given distilled water only; **B**: Given $NaAsO_2$ at 2.5 mg•kg^{-1} bw/day (p.o.) for 2 weeks; **C**: Administered COLEX alone at 200 mg•kg^{-1} bw a day for 2 weeks (p.o.); **D**: Pre-treated with COLEX for 2 weeks followed by $NaAsO_2$. The activities of the enzymes aspartate and alanine aminotransferases (AST and ALT), alkaline phosphatase (ALP) and γ-glutamyl transferase (γGT) were determined in the treated and control animals as indices of hepatotoxicity.
Place and Duration of the Study: The animal treatment and analyses were carried out at Department of Biochemistry, University of Ibadan between February and June 2008.

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Results: At 25, 40 and 50 μg•ml^{-1} concentrations of the extracts or the antioxidants, the reducing power is of the order BHA > BHT > COLEX. At 50 μg•ml^{-1}, the percentage inhibitions of peroxidation by COLEX, BHA and BHT were respectively 96.2%, 97.3% and 98.4% while percentage DPPH scavenging effect of COLEX, BHA and BHT were 62.5%, 67.5% and 61.3% respectively. The H_2O_2 scavenging activities were respectively 53.0%, 85.3% and 97.8% for COLEX, BHA and BHT. Pre-treatment with COLEX before administration of $NaAsO_2$ led to significant ($p < 0.05$) reduction in the mean liver and serum γGT, and serum ALP and AST activities when compared with group administered only $NaAsO_2$.
Conclusion: COLEX exhibited hepatoprotective effects against $NaAsO_2$ toxicity in male rats.

ABBREVIATIONS

γGT: γ-glutamyl transferase; ALP: Alkaline phosphatase); BHA: Butylated hydroxyanisole; BHT: Butylated hydroxytoluene; COLEX: Ethanolic extracts of Cassia occidentalis leaves.

1. INTRODUCTION

Human population is exposed to a number of chemicals either accidentally, occupationally or through life style habits. Many of these foreign substances exhibit their toxic effects by generating reactive oxygen species and other free radicals which cause damages to cells and various cellular molecules. The reactive oxygen species are causative factors in the aetiologies of many diseases such as cancers and hepatopathies [1-3]. The crucial role of the liver in the metabolism of foreign chemical substances in the body makes it one of the organs evoking toxic responses and an important target for toxicological studies.

A significant environmental contaminant is sodium arsenite. It is a component of herbicides, fungicides, insecticides, algaecides and arsenical soap [4-7]. Drinking water with high inorganic arsenic concentration is the main source of arsenic exposure for the world population [8]. Many studies have proved sodium arsenite to be highly toxic [9-11]. Other studies have shown that sodium arsenite is hepatotoxic and clastogenic in experimental animals [12-14].

Dietary intervention and the use of natural products, as found in the medicinal plants, offer possible means of control of the incidence of food/contaminants related diseases [15-16]. *Cassia occidentalis* L is a plant that has been widely employed in indigenous and folk medicine in the treatment of a wide range of ailments such as antidote of poison, blood purifier, expectorant, anti-inflammatory agent and a remedy for the treatment of liver diseases [17-18]. It is used in various liver conditions such as anaemia, hepatitis and liver damage. It could possibly help prevent liver cells damage. Water-ethanolic extract of Nigerian *C. occidentalis* leaf have been shown to contain alkaloids, tannins, saponins and phlobatannins [19]. Ethnobotany, phytochemical, pharmacological and toxicological activities of *C. occidentalis* have been comprehensive reviewed by Yadav et al. [20]. In the present study, we investigated (i) *in vitro* antioxidant/ radical scavenging activities of ethanolic extracts of Cassia occidentalis leaves (COLEX) in comparison with standard antioxidants,

butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); and (ii) its hepatoprotective ability of male Wistar rats treated with sodium arsenite.

2. MATERIALS AND METHODS

2.1 Chemicals

Sodium arsenite, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) were purchased from Sigma Chemical Co., St Louis, MO, USA. Kits for ALT, AST, ALP and γGT were purchased from Randox Laboratories Ltd (UK). All other reagents and chemicals were of analytical grade, products of Sigma Chemical Co, or BDH Chemical Ltd, Poole, England.

2.2 *Cassia occidentalis* and Extraction Procedures

Fresh *Cassia occidentalis* leaves were collected from the Botanical Garden, University of Ibadan and were authenticated at the Herbarium, Department of Botany, University of Ibadan. The leaves were dried at room temperatures on the laboratory bench surface. The dried leaves were ground into a fine powder with an electric blender and extracted with 70% ethanol solution. Extraction methods used were according to Gulcin [21]. Thereafter, the solvent were removed using rotary evaporator. The extract was placed in a plastic bottle and stored at -20ºC until used.

2.3 Antioxidant and Radical Scavenging Activities of *Cassia occidentalis* Leaves Extract

2.3.1 Total reductive capacity

The reductive capacity of COLEX was determined by the method of Oyaizu [22]. This method was described comprehensively by Gulcin et al. [21]. Briefly, to 1 ml of COLEX, BHA and BHT (each at 25, 40 and 50 $\mu g \cdot ml^{-1}$), were added 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide, $[K_3Fe(CN)_6]$. The mixture was incubated at 50ºC for 20 min. 2.5 ml of 10% trichloroacetic acid were added to each of the mixture which was then centrifuged at 1000 x g. The upper layer of the mixture was pipetted out and mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$. Absorbance was measured at 700 nm in a Spectrumlab 23A spectrophotometer (Techmel & Techmel Texas USA). Increased absorbance of the reaction mixture indicates increased reducing power.

2.3.2 Total antioxidant activity determination by ferric thiocyanate method

The method of Mitsuda et al. [23] with the modifications described by Gulcin et al. [24] was employed for the determination of total antioxidant activity of ethanolic extract of *Cassia occidentalis* leaves (COLEX) and standards antioxidant compounds (BHA and BHT). For each, a stock solution of 1 $mg \cdot ml^{-1}$ distilled water was prepared. Then, each of the solution containing different concentrations (25, 40 and 50 $\mu g \cdot ml^{-1}$) of the extract or the standards in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 ml of linoleic acid emulsion. The linoleic emulsion (5 ml) contained 17.5 μg Tween 20, 15.5 μl linoleic acid and 0.04 M potassium phosphate buffer. On the other hand, the control was composed of 2.5 ml potassium phosphate buffer and 2.5 ml of linoleic acid emulsion. The mixed solutions were incubated at 37ºC in a glass flask in the dark. The peroxide levels were determined by reading the absorbance at 500 nm in a Spectrumlab 23A spectrophotometer (Techmel &

Techmel Texas USA) after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. These steps were repeated until the control reached its maximum absorbance value. The percentage inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following:

$$\text{Inhibition of lipid peroxidation (\%)} = 100 - \left[\frac{A_{Control} - A_{Sample}}{A_{Control}} \right] \times 100$$

Where A_{sample} is the absorbance in the presence of the extract COLEX or standard compound BHA or BHT and $A_{control}$ is the absorbance of the control reaction mix.

2.3.3 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity

The scavenging capacity of free radical (DPPH) by COLEX, BHA and BHT were evaluated as described by Elmastas [25]. This involves the monitoring of the bleaching rate of the stable free radical DPPH at the characteristic wavelength (517 nm) in the presence or absence of the sample. An antioxidant would decrease DPPH absorbance [25]. A 1 ml solution (0.1 mM) of DPPH in ethanol was added to 3 ml of different concentrations (25, 40 and 50 µg/ml) of COLEX, BHA or BHT. The absorbance was read at 517 nm after 30 minutes. A lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capacity to scavenge DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}} \right] \times 100$$

Where $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of the sample (COLEX, BHA or BHT).

2.3.4 Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of COLEX was determined according to the method of Ruch et al. [26]. A 40 mM solution of H_2O_2 was prepared in phosphate buffer (pH 7.4). Extracts or standard (50 µg•ml^{-1}) was added to 0.6 ml of the H_2O_2. The absorbance of the mixture was read at 230 nm. Blank solution contained the buffer without H_2O_2. The percentage H_2O_2 scavenged by the COLEX, BHA and BHT was calculated as follows:

$$H_2O_2 \text{ scavenged activity (\%)} = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}} \right] \times 100$$

Where $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of the sample (COLEX, BHA or BHT).

2.4 Experimental Animals and Treatments

Male Wistar rats approximately thirteen weeks old and in the weight range of (130 – 180) g, healthy and with no injury were obtained from the Animal House of the Faculty of Basic Medical Sciences. They were housed in steel metal cages in the Animal House of Department of Biochemistry. The rats were divided into four groups (A - D) of 5 animals each, based on the treatment received. *Cassia occidentalis* leaves extract was suspended in convenient volume of distilled water and administered as indicated below.

- **A** : Group of rats given distilled water only.
- **B** : Animals in this group were given sodium arsenite at 2.5 mg•kg^{-1} body weight/day (p.o.) for 2 weeks.
- **C** : Animals in this group were given *Cassia occidentalis* leaf extract alone by oral at 200 mg•kg^{-1} body weight a day for 2 weeks by oral intubation.
- **D** : This group were pre-treated with *Cassia occidentalis* leaf extract (p.o.) at 200 mg•kg^{-1} body weight/day for 2 weeks followed by sodium arsenite at 2.5 mg•kg^{-1} body weight for 2 weeks.

All the rats were fed rodent pellets and given drinking water *ad libitum*. The rats were allowed to acclimatise for seven days before the commencement of the experiment and kept at 12 hours light/ dark cycle and temperature of 29 ± 2ºC throughout the duration of the experiment. Following the above treatment, the animals were fasted 24 hours before they were sacrificed by cervical dislocation.

2.4.1 Enzyme assays

From each of the sacrificed animals, serum was separated from blood that has been allowed to clot at room temperature (for about two hours). The clotted blood samples were centrifµged at 3,000 x g for 10 minutes; the supernatant (i.e. the serum) was separated and used immediately or stored at -20ºC until required. Livers were washed in phosphate buffered saline, blotted dry on filter paper and weighed. The liver was homogenized in 4 volume of phosphate buffered saline. The homogenate was centrifuged at 12,000 x g for 20 minutes and the supernatant stored at -20ºC.

2.4.2 Gamma-glutamyl transferase (γGT) activity

γGT was assayed in the serum and liver homogenates by using the reconstituted γGT diagnostic reagent following the method of Szasz [27].

2.4.3 Alanine aminotransferase (ALT) and aspartate aminotransferase(AST) activities

Serum ALT and AST were assayed according to Reitman and Frankel [28]. This method involves the reaction of pyruvate, the product of transamination reaction catalysed by ALT or AST, with 2, 4-dinitrophenyl hydrazine to produce intensely coloured hydrazone read at 546 nm using spectronic-20 spectrophotometer.

2.4.4 Alkaline phosphatase (ALP) activity

Serum ALP assay was based on the method of Williamson [29]. This involves a spectrophotometric (405 nm) determination of concentration of p-nitrophenol formed by the dephosphorylation of p-nitrophenyl phosphate (PNPP) catalysed by ALP.

2.4.5 Statistical analysis

The data were analysed by One-way Analysis of Variance (ANOVA) followed by analysis of Least Significant Difference (LSD). Values were considered statistically significant when P=0.05. All the results are expressed as mean $\pm$ standard deviation.

3. RESULTS

3.1 Antioxidant and Radical Scavenging Activities of *Cassia occidentalis* Leaves Extract

Total reducing capability, total antioxidant activity, DPPH radical and hydrogen peroxide scavenging assays were used for the evaluation of antioxidant activities of ethanolic extract of *Cassia occidentalis* leaves. It was found that the reducing ability of the COLEX and standard compounds, BHA and BHT increased with increasing concentrations. At all concentrations, the reducing power is in the order BHA > BHT > COLEX (Fig. 1). Secondly, the total antioxidant activity of COLEX, BHA and BHT were determined by ferric thiocyanate in linoleic acid emulsion method. There was increase absorbance at 500 nm with the increasing concentration of COLEX, BHA and BHT from 25 – 50 µg•ml^{-1} as shown in Fig. 2. The corresponding percentage inhibition of peroxidation in the linoleic acid system (calculated as in material and methods section) was found to increase steadily for COLEX, BHA and BHT with increasing concentration (Fig. 3). At 50 µg•ml^{-1}, the percentage inhibitions of peroxidation by COLEX, BHA and BHT were respectively 96.2%, 97.3% and 98.4% (Fig. 3). Furthermore, the antioxidant activities of COLEX, BHA and BHT at different concentrations were also determined by DPPH scavenging method. There were increased DPPH scavenging abilities with increasing concentration of COLEX and the standard compounds (Fig. 4). At 50 µg•ml^{-1} concentration, the calculated percentage scavenging effect of the extract, COLEX (62.5%), is similar to those of BHA and BHT (67.5% and 61.3 % respectively). Likewise, the hydrogen peroxide scavenging activities of COLEX, BHA and BHT were determined at 50 µg•ml^{-1} concentration in each case. The H_2O_2 scavenging activities were respectively 53.0%, 85.3% and 97.8% for COLEX, BHA and BHT (Fig. 5).
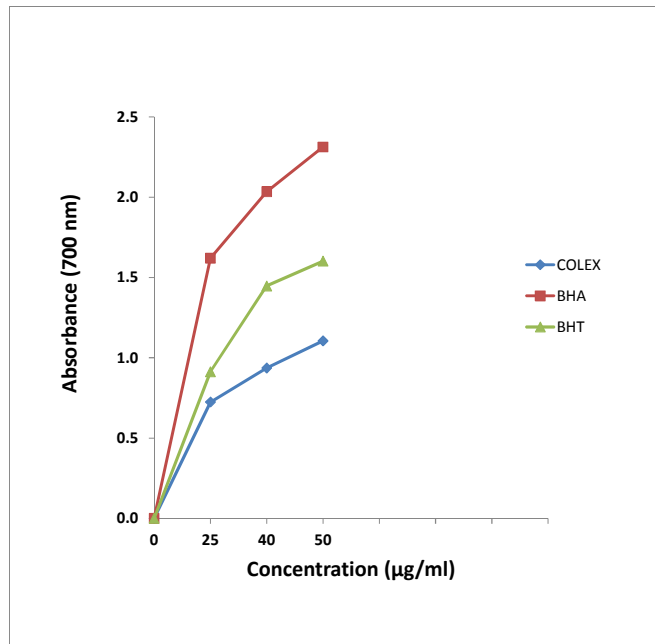
Fig. 1. Total reductive capability of COLEX, BHA and BHT at different concentrations using the potassium ferricyanide reduction method. COLEX is ethanolic extracts of *Cassia occidentalis* leaves; BHA (butylated hydroxyanisole); BHT (butylated hydroxytoluene). Indicated are the mean values for duplicate analyses
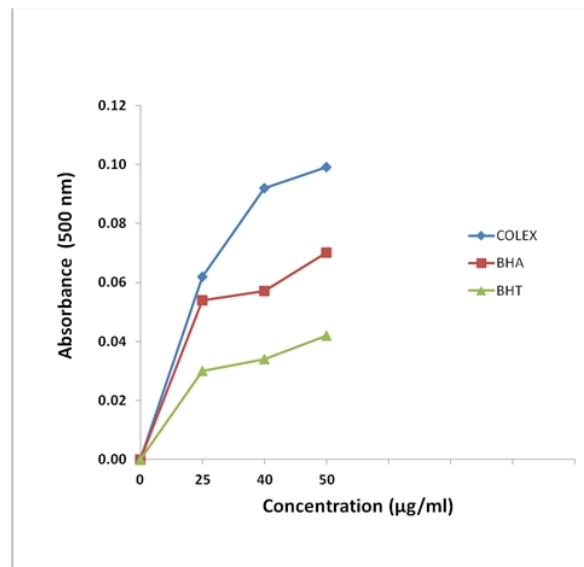

Fig. 2. Total antioxidant activities at different concentrations of COLEX, BHA and BHT using ferric thiocyanate in linoleic acid emulsion method. COLEX is ethanolic extracts of *Cassia occidentalis* leaves; BHA (Butylated hydroxyanisole); BHT (Butylated hydroxytoluene). Indicated are the mean values for duplicate experiments
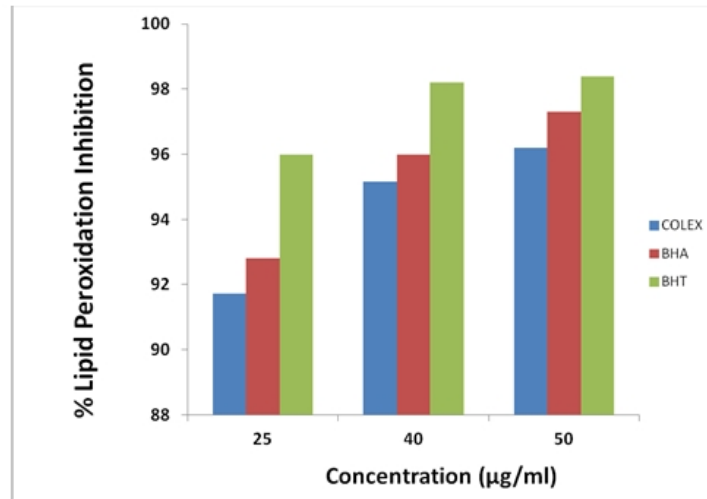
Fig. 3. Total antioxidant activities of COLEX, BHA and BHT at different concentrations measured as % lipid peroxidation inhibition. COLEX is ethanolic extracts of *Cassia occidentalis* leaves; BHA (Butylated hydroxyanisole); BHT (Butylated hydroxytoluene). Indicated are the mean values for duplicate analyses
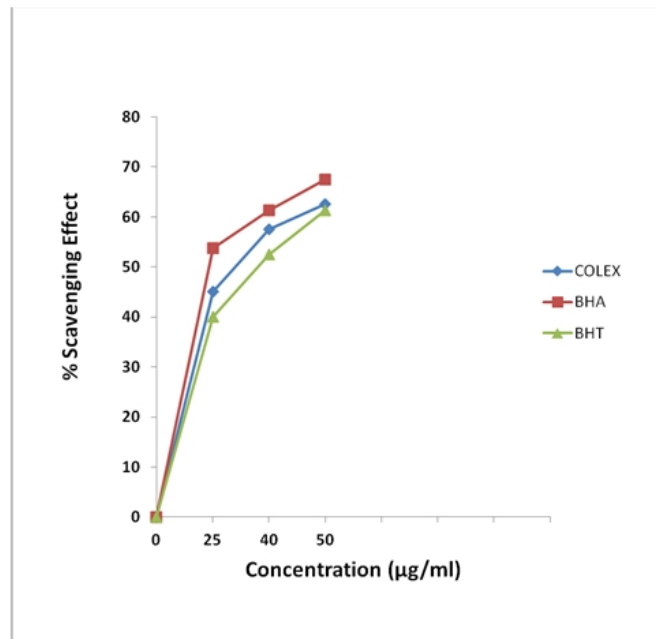

Fig. 4. Free radical (DPPH) scavenging ability of COLEX, BHA and BHT at different concentrations. COLEX is ethanolic extracts of *Cassia occidentalis* leaves; BHA (Butylated hydroxyanisole); BHT (Butylated hydroxytoluene). Values are means for duplicate analyses
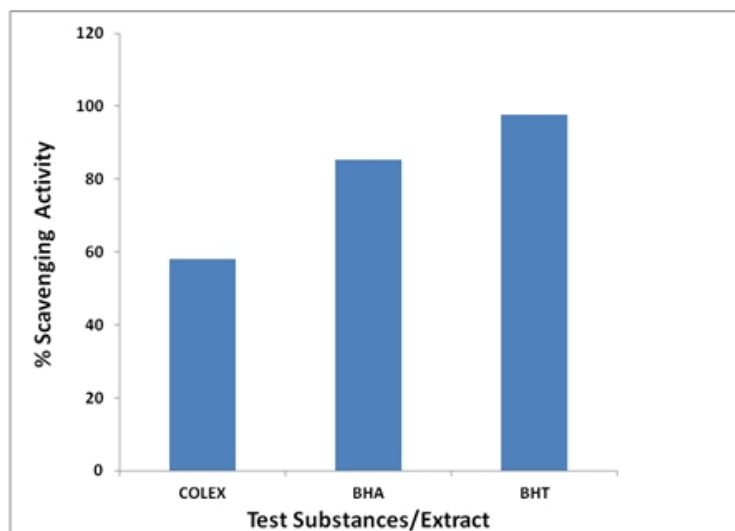
Fig. 5. Hydrogen peroxide scavenging ability of 50 µg•ml^{-1} concentration of COLEX, BHA and BHT. COLEX is ethanolic extracts of *Cassia occidentalis* leaves; BHA (Butylated hydroxyanisole); BHT (Butylated hydroxytoluene). Indicated are the mean values for duplicate analyses

3.2 Hepatotoxicity of Sodium Arsenite: Protective Effects of *Cassia occidentalis* Extracts

Experimental rats administered sodium arsenite (group B) recorded mean γ-glutamyl transferase (γGT) and alkaline phosphatase (ALP) activities that were significantly ($p < 0.05$) greater than the observation made with the negative control (group A) given distilled water (Figs. 6 and 7). Pre-treatment of the animals with extract of *C. occidentalis* resulted in significant ($p < 0.05$) reduction of the effect of sodium arsenite on the γGT and ALP activities (Figs. 6 and 7). Similarly rats administered sodium arsenite alone (group B) have elevated serum alanine and aspartate aminotransferase activities that are significantly ($p < 0.05$) different from the respective values obtained with the negative control group (Fig. 8). Treatments of the animals with ethanolic extract of *C. occidentalis* before the administration of sodium arsenite resulted in the attenuation of the sodium arsenite induction of these serum enzymes (group D) to levels that are significantly ($p < 0.05$) lower than group given sodium arsenite alone. However, the observed enzyme activities made with animals pre-treated with the *C. occidentalis* extract before the toxin are in all cases significantly ($p < 0.05$) higher that the negative control group (group D vs A, Figs. 6, 7 and 8).
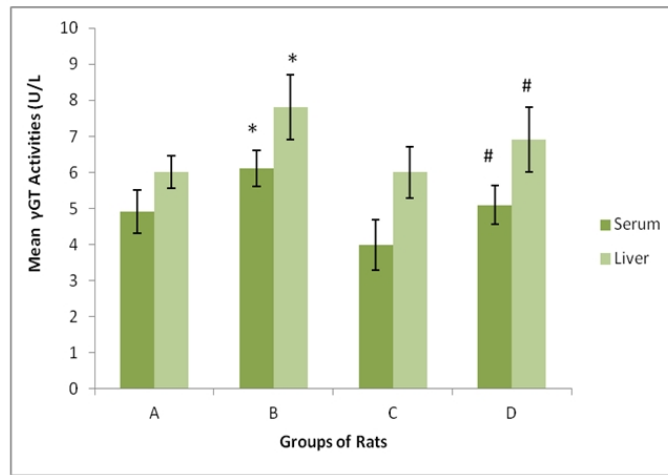
Fig. 6. Effect of ethanolic extracts of *Cassia occidentalis* leaves (COLEX) on sodium arsenite induction of liver and serum γ-glutamyl transferase (γGT)

*A: Control group of rats given distilled water only. **B**: Group of rats given sodium arsenite at 2.5 mg•kg-1 body weight/day (p.o.) for 2 weeks (positive control); **C**: animals in this group were given Cassia occidentalis leaf extract alone at 200 mg•kg-1 body weight a day for 2 weeks by oral intubation **D**: This group were pre-treated with Cassia occidentalis leaf extract at 200 mg•kg-1 body weight/day for 2 weeks followed by sodium arsenite at 2.5 mg•kg-1 body weight for 2 weeks. Values are Mean ± SD; n=5. *Significantly different from control group **A** (p < 0.05). #Significantly (p < 0.05) different from positive control group **B**.*
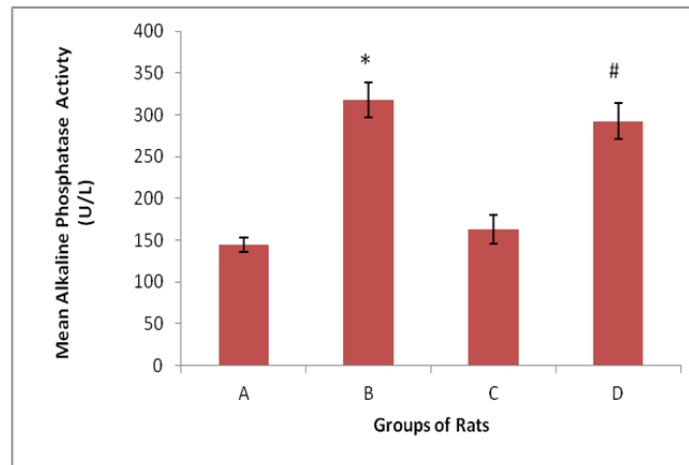

Fig. 7. Effect of ethanolic extracts of *Cassia occidentalis* leaves (COLEX) on sodium arsenite induction of serum alkaline phosphatase

*A: Control group of rats given distilled water only. **B**: Group of rats given sodium arsenite at 2.5 mg•kg-1 body weight/day (p.o) for 2 weeks (positive control); **C**: animals in this group were given Cassia occidentalis leaf extract alone at 200 mg•kg-1 body weight a day for 2 weeks by oral intubation **D**: This group were pre-treated with Cassia occidentalis leaf extract at 200 mg•kg-1 body weight/day for 2 weeks followed by sodium arsenite at 2.5 mg•kg-1 body weight for 2 weeks. Values are Mean ± SD; n=5. *Significantly different from control group **A** (p < 0.05). #Significantly (p < 0.05) different from positive control group **B**.*
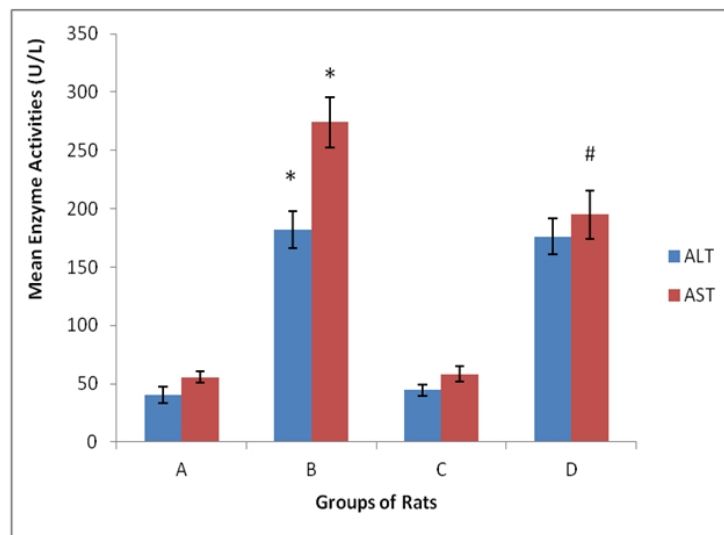
Fig. 8. Effect of ethanolic extracts of *Cassia occidentalis* leaves (COLEX) on sodium arsenite induction of serum amino transferases (ALT and AST)

A: Control group of rats given distilled water only. B: Group of rats given sodium arsenite at 2.5 mg•kg[-1] body weight/day (p.o) for 2 weeks (positive control); C: animals in this group were given Cassia occidentalis leaf extract alone at 200 mg•kg[-1] body weight a day for 2 weeks by oral intubation D: This group were pre-treated with Cassia occidentalis leaf extract at 200 mg•kg[-1] body weight/day for 2 weeks followed by sodium arsenite at 2.5 mg•kg[-1] body weight for 2 weeks. Values are Mean ± SD; n=5. *Significantly different from control group A (p < 0.05). #Significantly different (p < 0.05) from positive control group B.

4. DISCUSSION

Free radicals are causative factors in the aetiologies of many diseases [2-3]. They are easily generated through normal metabolic pathways in the body. Antioxidants, on the other hand are able to quench free radicals thereby preventing diseases. Natural antioxidants of plant sources have been associated with reduction of chronic diseases through their capacity to terminate free radical propagation in biological systems [30]. This capacity is widely used as a parameter to characterise nutritional and medicinal values of plants and their bioactive components.

In the present study, we have determined the antioxidant and free radical scavenging activities of ethanolic extract of *Cassia occidentalis* leaves (COLEX) in comparison with the standard antioxidants, BHA and BHT using four different methodologies including total reducing capability, total antioxidant activity, DPPH radical and hydrogen peroxide scavenging assays. In addition, we assessed the effect of COLEX on the sodium arsenite-induced hepatotoxicity in the male Wistar rats.

Potassium ferricyanide reduction method was used to determine the reducing power of COLEX, BHA and BHT. The reducing power of a compound serves as an indicator of its potential antioxidant activity. The reduction of Fe^{3+}/ferricyanide complex to the ferrous form is proportional to the power of the antioxidant substances present in the samples. The Fe^{2+}

formed as Perl's Prussian blue is then monitored at 700 nm [31]. The reducing ability of the samples is concentration dependent, increases with increasing concentration of the extract and the standards. At 50 µg•ml^{-1} concentration, the reductive power of COLEX is approximately 69% of BHT and 48% of BHA values (Fig. 1). The total antioxidant activity of *Cassia occidentalis* leaves extract, COLEX and the reference compounds, BHA and BHT (determined by percentage inhibition of lipid peroxidation) with ferric thiocyante method showed that the extract compared very well with the reference compounds (Fig. 3). The percentage inhibition calculated were of the order BHT > BHA > COLEX.

However, the percentage DPPH scavenging effect of COLEX, BHA and BHT were found to be very similar. A close look at the results of this assay indicates that COLEX was more efficient than BHT in scavenging DPPH radicals while BHA superseded COLEX as DPPH scavengers (Fig. 4). On the other hand, assay of hydrogen peroxide scavenging activities proved BHA and BHT to be more efficient than COLEX (Fig. 5). Aqueous extract of leaves of *C. occidentalis* have been shown to be most effective as free radical scanvenger in comparison with methanolic, chloroform, petroleum ether and benzene extracts [32]. The extensive antioxidant/ free radical activities of COLEX could be linked with the phytochemicals such as alkaloids, tannins, sapponins and phlobatannins identified in the water-ethanolic extract of Nigerian *C. occidentalis* leaves [19]. Moreover, recent studies have demonstrated that the phenolic compounds were responsible for the antioxidant potency of leaf and stem barks extracts of *C. occidentalis* and some medicinal plants [32-33]. Antioxidant properties, most especially free radical scavenging abilities, of components of food sources are very important due to the deleterious role of free radicals in food and in biological systems. Excessive generation of free radical accelerates the oxidation of lipids and other macromolecules in foods thereby decreasing food quality and consumer acceptance [34-37]. In addition, free radicals have been linked with many chronic pathological conditions [38-44]. On the other hand, effective food sourced antioxidant systems are able to prevent the above deleterious processes.

Short term exposure to sodium arsenite has been shown to induce hepatotoxicity in experimental animals [12-13,45] but mechanism of its hepatotoxicity is still under investigation. In the present study, we found experimental rats treated with sodium arsenite (group B) have mean liver and serum γGT, serum ALP, AST and ALT activities that are significantly higher (p < 0.05) than the value observed for the negative control group (group A). This is consistent with findings with other hepatotoxins [46-47]. In other words, the elevation in the level of these enzymes is an indication of liver lesion [48-50]. On the other hand, we found that the ethanolic leaf extracts of *C. occidentalis* attenuates the sodium arsenite-induced enzymes. Pre-treatment with leaf extracts of *C. occidentalis* before administration of sodium arsenite led to significant (p < 0.05) reduction in the mean liver and serum γGT and serum ALP and AST activities when compared with groups administered only sodium arsenite, supporting the presence of hepatoprotective components in the extracts against the hepatotoxicity of sodium arsenite.

Hepatotoxicity of sodium arsenite has been linked with its ability to generate free radicals *in vivo* [14,51-52]. It is possible that COLEX is able protect against sodium arsenite induced hepatotoxicity in the experimental animals because of its antioxidant and free radical scavenging activities. The findings here give scientific support and allude to the indigenous and folk medicine use of *Cassia occidentalis* L as a remedy for the treatment of liver diseases [17-18]. These findings are also in line with reports on extracts from other plants in rendering protective effects against chemicals and toxins [53-55].

5. CONCLUSION

Findings from this study revealed that ethanolic extract of *Cassia occidentalis* leaves possesses potent antioxidant and free radical scavenging activities. The extract also exhibited protective effects against sodium arsenite-induced hepatotoxicity in male rats.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), as well as University of Ibadan ethics committee rules on the use of laboratory animals in research were followed.

COMPETING INTERESTS

Authors have declared that there are no competing interests.

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