EFFECTS OF ROASTED *SENNA OCCIDENTALIS* SEEDS ON THE HAEMATOLOGY, HEPATORENAL FUNCTIONS AND HISTOPATHOLOGY OF ALBINO RATS

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ABSTRACT

The objective of this study is to evaluate the toxicological effects of aqueous extract of roasted Senna occidentalis seeds in rats. The acute toxicity was performed according to Lorke's method. For the evaluation of the effects of roasted S. occidentalis seeds on the haematology, hepatorenal functions and organ histopathology of albino rats, 24 adult male and female rats were divided into four groups of twelve (six males and six female) rats each respectively as follows: 1 (control group given only water), 2 (0.1.0% S. occidentalis seed powder), 3 (0.5% S. occidentalis seed powder), and 4 (1.0% S. occidentalis seed powder). Changes in body weight and food consumption as well as death were observed. In addition, the haematology, kidney and liver function, as well as the histological features, were evaluated. The results showed that S. occidentalis roasted seed extract did not cause mortality or behaviour alterations in the rats. There were no significant changes in body weight, food consumption, biochemical and haematological parameters as well as tissue histology in the test groups compared to the control rats. Based on these findings, it can be concluded that S. occidentalis roasted seed extract did not cause significant changes in most of the parameters evaluated, suggesting its potential safety for consumption.

Keywords: *Senna occidentalis,* Rats, Haematology, Liver and kidney functions, Organ histopathology

INTRODUCTION

The consumption of plant-based beverages, especially herbal teas, has gained recognition for their potential health-promoting effects. The plant *Senna occidentalis* L. (Fabales: Fabaceae), commonly known as coffee senna, is found in waste places, open pastures and fields cultivated with economic crops such as soybean, cotton, corn and sorghum (Adekunle *et al.,* 2018). Coffee senna seeds are consumed mainly as substitutes for coffee when they are roasted and constituted into a coffee-like beverage (Abubakar and Sule, 2010; Sharma *et al.,* 2013; Shittu *et al*., 2014; Teles *et al*., 2015; Adekunle *et al.,* 2018). The roasting of its seeds is a primary stage of preparation intended to alter sensory properties; and improve palatability, tastes, aromas and textures. Roasting also helps to inactivate microbes and enzymes, as well as physical and chemical changes that may cause toxicity (Brennan *et al*., 2006; Belitz *et al.,* 2009).

The tissues of *S. occidentalis* contain various phytoactive chemicals that support its use in folk medicine. The extracts or powdered leaves have been traditionally used as analgesics, antibacterials, antifungals, anti-inflammatories, antiseptics, antispasmodics, antiparasitic, antivirals,

diaphoretics, insecticides, laxatives, purgatives, and for the treatment of stomach disorders, rheumatism, and liver diseases (Di Stasi and Hiruma-Lima, 2002). It is distributed as a weed throughout tropical and subtropical regions of the world, especially in West Africa where its seeds are commonly roasted and infused to make a coffee-like beverage (Medoua and Mbofung, 2007).

However, studies have indicated that *S. occidentalis* may be responsible for a syndrome characterized by generalized muscle degeneration (Tasaka *et al*., 2000). Despite this potential toxicity, the beverage made from *S. occidentalis* seeds is widely consumed by populations in several West African countries (Medoua and Mbofung, 2007).

In a study from West Africa, the source of toxicity was found to be the consumption of *S. occidentalis* beans by unsupervised young children from low-income families (Panigrahi *et al.,* 2014). Preclinical studies on the effect of roasting on the haematological and hepatorenal toxicity of the seeds of *S. occidentalis* on albino rats abound. Thus, there is a need to confirm the potential toxicity of roasted seeds of *S. occidentalis* to determine their safety for human consumption. This study aims to investigate the sub-acute toxicity effects of *S. occidentalis* roasted seed on the haematology, kidney and liver function, as well as the histological features of Wistar rats.

MATERIALS AND METHODS

Chemicals, Assay Kits and Reagents: The serum biochemistry assay kits were sourced from Quimica Clinica Applicada (QCA), Spain and Randox Laboratories Limited, County Antrim, United Kingdom. All chemicals and reagents used were of analytical grade.

Collection, Identification of Plant Material and Preparation of Extract: The fresh seeds of *S. occidentalis* were obtained from Mbamba, Adamawa State, Nigeria. The seed was identified (Utteridge and Bramley, 2015) and authenticated at the Department of Botany, Modibbo Adama University, Yola where a voucher specimen (MAU/2023/0012SE) was kept for referral purposes. The fresh seeds were thoroughly washed and dried at 27°C. Subsequently, the well-dried seeds underwent heat treatment by roasting at 200°C for 10 minutes in a hot air oven. The resulting seeds were then crushed into a coffee-like powder using a mechanical grinder. To prepare the Senna Tea, various concentrations of 0.0, 0.1, 0.5 and 1.0% (w/v) were created by adding the appropriate amount of boiling water to the roasted seed powder. The mixture was allowed to steam for 30 minutes and was then filtered using muslin cloth. The resulting suspension served as the beverage for the experiment (Kumar and Bhattacharya, 2008).

Experimental Animals: Forty-eight male and female albino rats weighing 100 ± 20 g were obtained from the National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State. These rats were housed in ventilated plastic cages and acclimatized for seven days before randomization into different groups. Throughout the experiment, the rats were provided with a standard dry pellet diet (Vital Feeds, Jos) and water *ad libitum*.

Experimental Design: The study was conducted following the Organization for Economic Cooperation and Development (OECD) test guidelines for repeated dose for 28 days (subacute) oral toxicity study in rodents. Twenty-four male and female albino rats were allocated to four treatment groups, replicated thrice with each replicate having two male and female rats respectively and administered the roasted seed beverage in drinking water at 0.0. 0.1, 0.5 and 1.0% for 28 days. The controls were administered with portable water only as shown in Table 1.

Sample Collection: At the termination of treatment, blood was collected from all animals by the orbital technique (Bolliger and Everds, 2010). The blood collected was divided and dispensed into two sample bottles containing ethylene diamine tetra-acetic acid (EDTA) for haematology and the other without EDTA for serum collection.

Haematology: Red blood cell (RBC) and white blood cell (WBC) counts were determined by the haemocytometer method (Thrall and Weiser, 2002).

Table 1: Experimental design on the effect of *Senna occidentalis* roasted seed extract in albino rats

Group	Treatment	Number of animals		Study duration
		Male	Female	
Group 1	Drinking water only (control)	6	6	28 days
Group 2	0.1% of *Senna occidentalis* seed powder in drinking water	6	6	
Group 3	0.5% of *Senna occidentalis* seed powder in drinking water	6	6	
Group 4	1.0% of *Senna occidentalis* seed powder in drinking water	6	6	

Packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002), while the haemoglobin (Hb) concentration was determined by the cyanomethaemoglobin method (Higgins *et al.,* 2008a).

Serum Biochemistry: Liver enzyme activities such as serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the Reitman-Frankel method (Reitman and Frankel, 1957), while serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Ochoa, 1968), serum total bilirubin was determined by the Jendrassik-Grof method (Higgins *et al.,* 2008b). Serum total protein was determined by the direct Biuret method, while serum albumin was determined by the bromocresol green method (Busher, 1990). The serum globulin was calculated as the difference between the serum total protein and serum albumin. The serum urea was determined by the modified Berthelot-Searcy method (Lamb and Price, 2008), while the serum creatinine level was determined by the modified Jaffe method (Lamb and Price, 2008).

Histopathology: The rats were weighed after blood sample collection, and humanely sacrificed by intraperitoneal injection of 250 mg/kg sodium thiopentone (Underwood *et al*., 2013). Thereafter, the animals were dissected and the liver and kidneys were removed and fixed in 10.0% buffered formalin. Five-micrometre thick sections were cut, processed, mounted on glass slides and stained with Hematoxylin and Eosin for light microscopy (Nikon, Tokyo, Japan). Photomicrographs of the sections were captured using Motic Images plus a 2.00 digital camera (Motic, Xiamen, China).

Statistical Analysis: The data were analyzed using one-way analysis of variance (ANOVA). Significant means were separated using a post-hoc test ($p<0.05$). All analyses were done using SPSS version 26.0 and the results were expressed as means ± standard error of the mean.

RESULTS

Effects of *Senna occidentalis* Roasted Seed Extract on the Body Weight of Rats: Table 2 shows the effect of *S. occidentalis* roasted seed extract on the body weight of rats. The administration of *S. occidentalis* roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not significantly alter ($p>0.05$) the percentage body weight gain and the final body weight in the male rats when compared with the control rats. More so, the administration of *S. occidentalis* roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not significantly alter ($p>0.05$) the final body weight of the female animals, but significantly reduced the body weight gain in female rats when compared with the control animals.

Effect of *Senna occidentalis* Roasted Seed Extract on Food and Water Consumption in Rats: Administration of *S. occidentalis* roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not show a significant difference ($p>0.05$) in food consumption of male rats. However, rats administered extracts at 0.5 and 1.0% showed significantly lower ($p<0.05$) water consumption in both males and females compared to the control and 0.5% groups (Table 3). More so, administration of *S. occidentalis* roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not show a significant difference ($p>0.05$) in food consumption in both male and female rats. However, food consumption was relatively higher in female rats administered extract at concentrations of 0.0, 0.1, 0.5 and 1.0% compared to the control group (Table 3).

Table 2: Effect of *Senna occidentalis* roasted seed extract on the body weight of male and female albino rats

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (%)
Males			
1: (control)	139.10 ± 5.73	229.80 ± 9.13	65.20
2: 0.1% of *Senna occidentalis*	142.90 ± 6.89	234.60 ± 9.45	64.20
3: 0.5% of *Senna occidentalis*	144.92 ± 3.97	236.80 ± 14.73	63.40
4: 1.0% of *Senna occidentalis*	144.89 ± 4.52	231.20 ± 10.74	59.60
Females			
1: (control)	113.90 ± 1.76	195.20 ± 9.18	71.38^{b}
2: 0.1% of *Senna occidentalis*	121.00 ± 7.20	198.80 ± 7.04	64.30^{a}
3: 0.5% of *Senna occidentalis*	116.40 ± 2.06	192.10 ± 5.75	65.03^{a}
4: 1.0% of *Senna occidentalis*	118.40 ± 3.14	195.80 ± 10.11	65.37^{a}

Values are represented as mean ± SEM, n = 6, ab = mean values on the same column with different letter superscripts are significantly different (p<0.05)

Table 3: Effect of *Senna occidentalis* roasted seed extract on food and water consumption of male and female Wistar rats

Groups	Food consumption (g/day)		Water consumption (ml/day)		Extract intake (mg/Kg BWT)
	Group	Individual	Group	Individual	
Males					
1: (control)	99.20 ± 1.01	16.53 ± 0.17	300.60 ± 1.55^{b}	49.90 ± 1.89	0.00
2: 0.1% of *Senna occidentalis*	94.90 ± 0.95	15.81 ± 0.16	303.40 ± 1.46^{b}	50.67 ± 1.92	268.45
3: 0.5% of *Senna occidentalis*	95.67 ± 10.60	15.95 ± 0.33	269.00 ± 0.08^{a}	44.89 ± 1.51	275.99
4: 1.0% of *Senna occidentalis*	97.77 ± 1.97	16.30 ± 0.33	258.00 ± 1s.88^{a}	43.11 ± 1.32	272.48
Females					
1: (control)	96.30 ± 1.99	16.05 ± 0.33	314.90 ± 1.41^{b}	52.50 ± 2.24	0.00
2: 0.1% of *Senna occidentalis*	99.08 ± 1.64	16.50 ± 0.27	273.00 ± 0.64^{a}	45.50 ± 1.63	284.55
3: 0.5% of *Senna occidentalis*	97.92 ± 1.86	16.32 ± 0.31	270.30 ± 2.48^{a}	45.14 ± 1.41	273.21
4: 1.0% of *Senna occidentalis*	101.40 ± 1.61	16.90 ± 0.43	269.00 ± 1.42^{a}	44.80 ± 1.06	271.69

Values are represented as mean ± SEM, n = 6, a – d = mean values on the same column with different letter superscripts are significantly different (p<0.05)

Effects of Roasted Seeds Extract of *Senna occidentalis* on Liver and Kidney Function Indices of Rats: Administration of *S. occidentalis* roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not significantly alter (p>0.05) AST, ALT, ALP, total protein, albumin and bilirubin in both male and female rats in the treatment groups when compared with control (Table 4). Also, administration of S. occidentalis roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not significantly alter (p>0.05) serum urea and creatinine concentration in both male and female rats in the treatment groups when compared with control (Table 4).

Effects of Roasted Seeds Extracts of *Senna occidentalis* on Some Haematological Parameters: Administration of *S. occidentalis* roasted seed extract at 0.0, 0.1, 0.5 and 1.0% did not significantly alter (p>0.05) WBC count, RBC count, PCV and Hb concentrations of both male and female rats in the treatment groups when compared with control (Table 5).

Histopathology: Histologic examination of sections of the liver and kidney of control and *S. occidentalis* treated groups showed the absence of microscopic lesions in both male and female rats (Figure 1).

Table 4: Effect of *Senna occidentalis* roasted seed extract *on* the liver and kidney function of male and female albino rats

Serum biochemistry parameters	Group 1 (control)	Group 2 0.1% of *Senna occidentalis*	Group 3 0.5% of *Senna occidentalis*	Group 4 1.0% of *Senna occidentalis*
Males				
Serum aspartate aminotransferase (IU/L)	28.30 ± 1.90	27.00 ± 0.97	27.70 ± 1.22	26.00 ± 1.10
Serum alanine aminotransferase (IU/L)	38.50 ± 2.72	38.00 ± 5.20	36.80 ± 4.20	36.00 ± 4.70
Serum alkaline phosphatase (IU/L)	134.00 ± 10.80	132.70 ± 13.30	133.70 ± 12.80	131.00 ± 11.58
Serum total bilirubin (mg/dL)	9.95 ± 2.30	9.60 ± 2.10	9.71 ± 2.40	9.10 ± 1.96
Serum direct bilirubin (mg/dL)	66.67 ± 2.60	66.00 ± 2.99	65.17 ± 3.30	66.17 ± 1.62
Serum total protein (g/dL)	66.70 ± 2.60	67.00 ± 3.90	63.30 ± 3.30	65.70 ± 1.80
Serum albumin (g/dL)	32.00 ± 0.58	32.20 ± 0.83	31.80 ± 0.60	30.00 ± 0.48
Serum creatinine (mg/dL)	159.70 ± 12.80	157.70 ± 11.40	157.30 ± 2.40	158.00 ± 9.30
Serum urea (mg/dL)	6.65 ± 0.20	7.07 ± 0.60	6.83 ± 0.32	7.68 ± 0.39
Females				
Serum aspartate aminotransferase (IU/L)	38.30 ± 6.04^{c}	28.80 ± 11.36^{a}	32.80 ± 19.57^{b}	31.50 ± 5.74^{b}
Serum alanine aminotransferase (IU/L)	45.50 ± 10.05^{c}	25.80 ± 4.79^{a}	39.17 ± 9.58^{b}	40.67 ± 1.84^{b}
Serum alkaline phosphatase (IU/L)	127.70 ± 8.99^{a}	140.00 ± 12.09^{b}	163.00 ± 18.99^{d}	154.70 ± 10.74^{c}
Serum total bilirubin (mg/dL)	15.50 ± 4.14^{c}	9.96 ± 2.26^{a}	18.02 ± 2.20^{d}	13.20 ± 0.63^{b}
Serum direct bilirubin (mg/dL)	1.35 ± 0.26	1.08 ± 0.16	1.50 ± 0.15	1.17 ± 0.08
Serum total protein (g/dL)	64.30 ± 1.26	63.80 ± 1.42	65.17 ± 1.28	65.67 ± 2.30
Serum albumin (g/dL)	31.30 ± 0.61	30.50 ± 0.42	31.50 ± 0.56	32.00 ± 0.68
Serum creatinine (mg/dL)	137.20 ± 3.98	141.00 ± 17.72	142.70 ± 12.08	123.50 ± 1.02
Serum urea (mg/dL)	7.80 ± 0.36	7.80 ± 0.45	7.60 ± 0.13	6.81 ± 0.32

Values are represented as mean ± SEM, n = 6, a – d = mean values on the same column with different letter superscripts are significantly different ($p<0.05$)

Table 5: Effect of *Senna occidentalis* roasted seed extract on some haematological parameters of male and female albino rats

Haematological parameters	Group 1 (control)	Group 2 0.1% of *Senna occidentalis*	Group 3 0.5% of *Senna occidentalis*	Group 4 1.0% of *Senna occidentalis*
Males				
Packed cell volume (%)	42.35 ± 1.78	39.93 ± 1.03	42.25 ± 0.86	41.52 ± 0.60
Haemoglobin concentration (g/dL)	14.60 ± 0.82	13.30 ± 0.35	14.20 ± 0.24	13.80 ± 0.20
Red blood cell count (10^6/µL)	7.40 ± 0.26	6.78 ± 0.17	6.94 ± 0.22	7.01 ± 0.12
white blood cell count (10^3/µL)	16.05 ± 1.94	16.43 ± 1.71	16.20 ± 1.61	15.77 ± 0.79
Females				
Packed cell volume (%)	40.78 ± 1.81	39.57 ± 0.69	38.50 ± 0.86	39.20 ± 1.35
Haemoglobin concentration (g/dL)	13.59 ± 0.60	13.40 ± 0.24	12.84 ± 0.29	13.17 ± 0.44
Red blood cell count (10^6/µL)	6.50 ± 0.16	6.61 ± 0.17	6.47 ± 0.09	6.43 ± 0.26
White blood cell count (10^3/µL)	13.70 ± 2.10	13.10 ± 2.30	12.90 ± 2.7	12.69 ± 1.00

Values are represented as mean ± SEM, n = 6, No significant differences between the means of the groups ($p>0.05$)

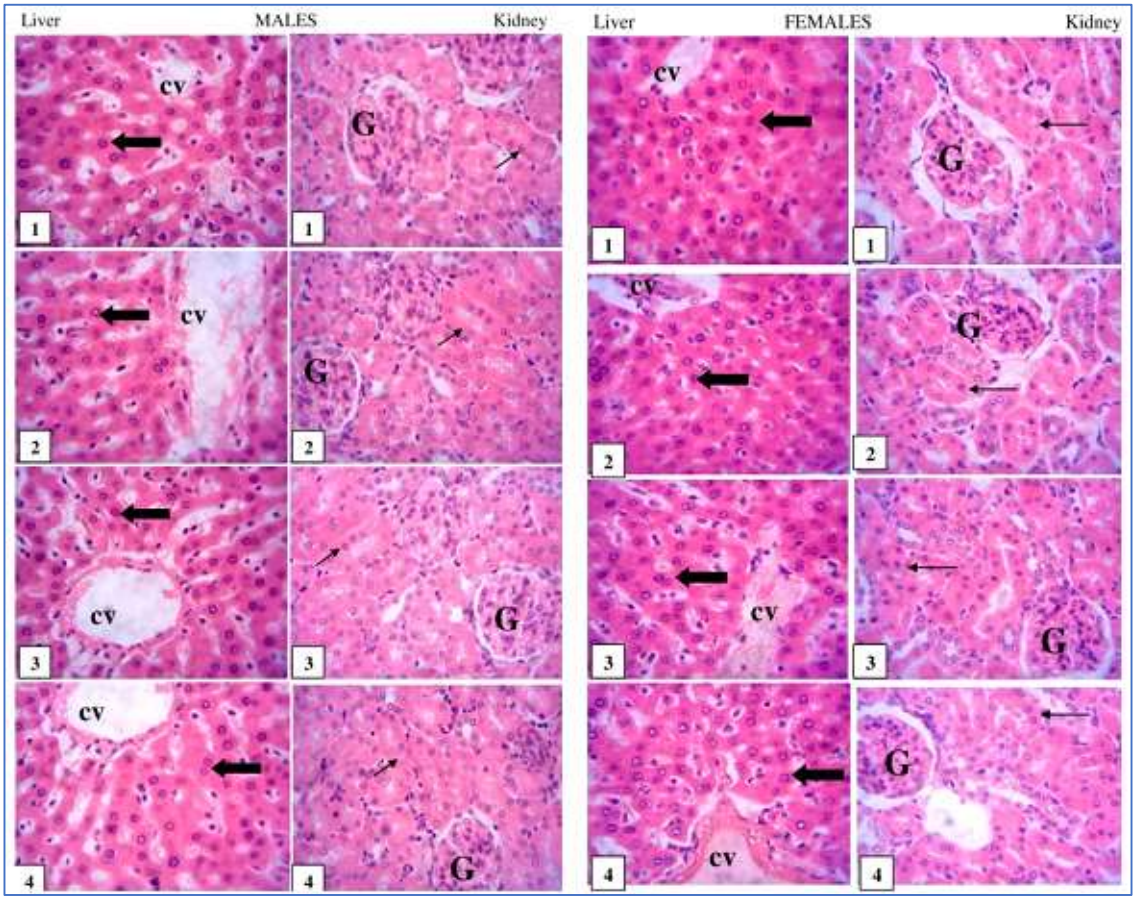

Figure 1: Photomicrograph of the liver and kidney sections of male and female rats in groups 1 (control), 2 (0.1% of *Senna occidentalis*), 3 (0.5% of *Senna occidentalis*) and 4 (1.0% of *Senna occidentalis*) showing normal histology (Note: CV = central vein; thick arrows = hepatocytes; G = glomerulus and thin arrows = renal tubules). H and E stain × 400

DISCUSSION

The results of the effects of *S. occidentalis* roasted seed extract on body weight which showed a significant reduction in body weight gain in female rats unlike in males; suggest that *S. occidentalis* roasted seed extract may have a differential effect on body weight gain in male and female rats. This is similar to a previous report which showed that females are more active than male rats (Eckel and Moore, 2004), leading to less weight gain seen in female rats. Food and water consumption patterns are important indicators of overall health and well-being. There was a significant reduction in water consumption observed in both sexes in the present study which is consistent with previous studies that have reported alterations in water intake following the administration of *S. occidentalis* extracts (Medoua and Mbofung, 2007). This was attributed to the fact that the beverage was not sweetened, thus resulting in reduced consumption.

The liver and kidneys play vital roles in maintaining homeostasis and overall health. In this study, the administration of *S. occidentalis* roasted seed extract did not significantly alter liver function parameters, such as AST, ALT, ALP, total protein, albumin, and bilirubin, in male or female rats. Similarly, there were no significant changes in kidney function parameters, including urea, creatinine, sodium, potassium, and chloride ions, in male or female rats. These findings suggest that *S. occidentalis* roasted seed extract was not toxic to the liver and kidney in rats. This correlates with the histopathology result which showed the absence of lesions in the liver and kidney of both sexes of rats. These results are

consistent with previous studies that have reported the safety of *S. occidentalis* in terms of liver and kidney functions (Silva *et al*., 2011; Mogaka *et al*., 2023).

Haematological parameters are important indicators of overall health and immune status. The absence of alterations in haematological parameters after administration of *S. occidentalis* roasted seed extract in male or female rats as observed in this study demonstrates the overall safety of *S. occidentalis* roasted seed extract. These results are consistent with previous studies that have reported the safety of *S. occidentalis* in terms of haematological parameters (Silva *et al*., 2011).

Conclusion: The findings of this study show that *S. occidentalis* roasted seed extract does not cause significant changes in most of the parameters evaluated, indicating its potential safety for consumption. Thus, this study has demonstrated that consumption of *S. occidentalis* roasted seed extract is safe, as evidenced by the absence of behavioural changes, mortality, alterations in liver and kidney function, haematological parameters, or histological changes in the tested rat models.

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