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Research article

Effects of Aqueous Leaf Extracts of *Senna occidentalis* on Rat Kidney

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ABSTRACT

Senna occidentalis is one of the most widely used herbal plants for various therapeutic purposes in Sokoto, Nigeria. Renal function and histopathological effects of aqueous leave extract of *Senna occidentalis* was investigated in Wistar rats. Preliminary phytochemical screening of the extract was carried out using standard procedures and this was followed by an acute toxicity study using Lorke's method. Twenty (20) rats were randomly selected and grouped into 5 groups of 4 rats each having a control group and 4 test groups. Aqueous leaf extract of *S. occidentalis* was administered orally to each test group at different concentrations of 350mg/kg, 700mg/kg, 1500mg/kg and 3000mg/kg body weight respectively for 28days. Blood samples were collected through cardiac puncture for electrolytes, urea and createnine biochemical analyses while the kidneys were collected through abdominal incision for histopathological analyses using haematoxylin and eosin staning method. Data generated was analyzed using GraphPad instat3 version 3.02 and presented as mean ± SEM, statistical comparison between groups were made using one-way analysis of variance (ANOVA) with post hoc Bonferroni Multiple Comparison Test. Phytochemical screening showed presence of carbohydrate, tannins, triterpenoids, proteins, saponins, steroids, flavonoids, diterpenoids and cardiac glycosides while alkaloids and anthraquinones were not detected. The animals did not show behavioral changed nor death even at a concentration of 5000mg/kg during acute toxicity testing. There were no significant effects on biochemical parameters between test groups and control. Histological sections of the kidney showed well-preserved glomeruli and tubules with only few animals showing mild to moderate sclerosis which is not dose-dependent. Oral administration of up to 3000mg/kg body weight of aqueous leaf extract of *Senna occidentalis* for 28 days did not produce significant effects on the kidneys of Wistar rats thereby suggesting its non-lethal effect on the kidneys.

Keywords: *Senna occidentalis, kidney, sub-acute administration, renal function*

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INTRODUCTION

Senna occidentalis is one of the most widely used herbal plants among people of tropical and sub-tropical regions of the world (Veronique and Gabriel, 2013). It is used for various therapeutic purposes in traditional medicine (Yadav *et al.*, 2010, Silva *et al.*, 2011). In Nigeria, this plant is locally called Sanga-sanga or Rai dore in Hausa language (Nuhu and Aliyu, 2008; Sadiq *et al.*, 2012); Akidi agbara in Igbo language and Abo rere in Yoruba language (Egharevba *et al.*, 2010). Roots, leaves, flowers and seeds of *Senna occidentalis* are the different parts of the plant used in medication (Veronique and Gabriel, 2013). The lethal dose (LD50) of aqueous leaf extract of *Senna occidentalis* was found to be safe up to 5000 mg/kg body weight (Silva *et al.*, 2011; Shafeen *et al.*, 2012; Tanimu and Wudil, 2012).

The plant has been used in different parts of the world by the traditional healers in treating different forms of diseases. It has been documented in literatures that extract of *Senna occidentalis* has antimicrobial activity (Mariano-Souza *et al.*, 2010; Mohammed *et al.*,2012), larvicidal and pupicidal activity (Ibrahim *et al.*, 2010), antioxidant and

hepatoprotective activity (Gowrisri *et al.,* 2012), anti-inflammatory actions (Yadav, 2010), antimalarial activity (Gwarzo *et al.,* 2014), antianxiety and antidepressant activity (Shafeen *et al.,* 2012), analgesic activity (Silva *et al.,* 2011) and antidiabetic activity (Emmanuel *et al.,* 2010; Laxmi *et al*., 2010; Onakpa and Ajagbonna, 2012).

According to World Health Organization (WHO), due to poverty and lack of access to modern medicines; about 65-80% of the world's populations living in developing countries depend essentially on plants for primary health care (Calixto, 2000). Traditional healers dispense herbal preparations without much consideration to the quantity of the extract ingested by their clients.

Despite the growing demand for herbal medicines, there are still concerns associated with not only their use, but also their safety (Winston and Maimes, 2007). Thousands of decades ago, people most especially rural dwellers relied heavily on traditional medicine using herbs for treatment of illnesses; this has been the practice in Northern Nigeria in particular Sokoto State and all over the world (Nuhu and Aliyu, 2008). The kidneys serve essential regulatory roles in animals by removing excess organic molecules from the blood as waste products of metabolism (Arthur and John, 2006; Inderbir, 2007). Kidneys are one of the vital organs affected by accumulation of toxic substances in the body; exposure to toxic substances can cause injury or death of tissues in the kidney resulting in leakage of essential biomolecules into the blood stream alongside with histomorphological changes (Vashishtha *et al.,* 2009; Nwaehujor *et al.,* 2011).

People consume *Senna occidentalis* extract either alone or mixed up with other mineral and organic matter as therapy (Garba *et al.,* 2015). The leaves of this plant are widely used in our community without enough knowledge of its possible side effects on vital organs. Therefore, this study sought to investigate the renal function and histopathological of the extract on Wistar rats.

MATERIALS AND METHODS

Plant Collection: Fresh leaves of *Senna occidentalis* were collected in the month of January, 2015 from Fadama site in Kwalkwalawa village, opposite main campus of Usmanu Danfodiyo University, Sokoto (130^0 21' 16''N and 50^0 5' 37''E) in a polythene bag. The plant was identified and authenticated at Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. A voucher of the plant specimen with number UDUH/ANS/OO23 was prepared and also deposited in the herbarium.

Extract Preparation: The leaves were washed thoroughly with tap water and rinsed finally with distilled water. The sample was then air dried at room temperature for 5days. After drying, the sample was grinded using blender and passed through 125µm sieve to obtain a fine powder. The dried powder was kept in an air-tight container.

The 200g of the dried powder was dissolved into two (2) litres of distilled water. The mixture was shaken at intervals for six (6) hours and then left to soak for 24 hours in a water bath set at 40^0c. The preparation was first filtered using a clean cloth to remove debris then re-filtered using Whatmann's filter paper. The resultant filtrate was concentrated to dryness at 40˚c under reduced pressure. The percentage recovery was calculated and the dried extract was stored in an air-tight container at 4^0c until use (Ibrahim *et al.,* 2010).

Phytochemical Screening: The aqueous extract of *Senna occidentalis* was subjected to qualitative test for the presence of bioactive components that include Molisch's test for detection of Carbohydrates, Meyer's test for detection of Alkaloids, Wagner's test for detection of Alkaloids, Lead sub-acetate test for detection of Tannins, Keller- Killiani's test for detection of Cardiac Glycosides, Frothing/Foaming test for detection of Saponins, Salkowkis's test for detection of Steroids and Triterpenoids, Libermann-Burchard's test for detection of Steroids and Triterpenoids, Copper Acetate's test for detection of Diterpenoids, Alkaline test for the detection of Flavonoids, Shinoda's test for the detection of Flavonoids, Xanthoproteic test for the detection of Protein, Borntrager's test for the detection of Anthraquinones **(**Sofowora, 1993; Trease and Evans, 2002; Adegoke *et al.,* 2010).

Experimental Animals: Thirty-seven (37) healthy Wistar rats were procured from Faculty of Veterinary Medicine of Ahmadu Bello University Zaria, Nigeria. The rats were housed in metal cages and kept in a well ventilated room with 12hours dark/light cycle. They were fed with standard feed pellets and tap water *ad libitum*. The animals were acclimatized for 2 weeks before proceeding with the experiment. The research was carried out in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2010)

Acute Toxicity Testing: Acute toxicity testing was conducted using Lorke's method (1983). In phase I, nine (9) albino rats were randomly assigned into 3 groups consisting of 3 rats each. The 1st group was administered 10mg/kg body weight of the extract while groups 2 and 3 were given 100mg/kg and 1000mg/kg respectively. The animals were then observed for 24 hours to monitor their behavior as well as any mortality. In Phase II, three (3) albino rats were randomly placed in 3 groups of 1 rat each. The rats were administered higher doses of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. They were then observed for 24 hours for behavioral change as well as mortality. All administrations were carried out using an oral cannula.

Experimental Design:

Twenty-five (25) albino rats were used for the experiment and distributed randomly into 1-5 groups with 5 rats per group.
Group 1: Each rat was given 350mg/kg of the extract
Group 2: Each rat was given 700mg/kg of the extract
Group 3: Each rat was given 1500mg/kg of the extract
Group 4: Each rat was given 3000mg/kg of the extract
Group 5: Each rat was given equivalent volume of distilled water.

Each rat in the test groups was served with volumes of the extract using an oral cannula according to their weight; those in the control group each was given equivalent volume of

distilled water and the administration of the plant extract lasted for 28 days respectively.

Samples Collection and Biochemical Analysis: Twenty-four (24) hours after the final administration of the extract, the animals were anaesthetized using chloroform vapour in an enclosed jar. Blood samples of 3 mls were collected through cardiac puncture into labeled plain tubes for biochemical analysis. The animals were then dissected through an abdominal incision to remove the kidneys which were subsequently immediately fixed in labeled 10% formol saline containers for histopathological analysis.

The blood samples collected in the plain containers are allowed to stand for 30 minutes and subsequently centrifuged at 3000rpm for 10 minutes. After the centrifugation, the serum (supernatant) was dispensed into labeled tubes using a Pasteur pipette and then subjected to kidney function test methodologies. The parameters analyzed are –

Sodium (Na) and Potassium (K) were analyzed using flame photometric method (Ochei and Kolhatkar, 2008).

Bicarbonate (HCO_3) was analyzed using titration method (Ochei and Kolhatkar, 2008).

Chloride (Cl) was analyzed using spectrophotometric method (Agappe, 2008).

Urea was analyzed using diacetyl monoxime method (Ochei and Kolhatkar, 2008).

Creatinine was analyzed using Japhet's method (Ochei and Kolhatkar, 2008).

Histopathological Analysis: The organs were brought out of the fixative and examined macroscopically on a cutting bench; representative parts of the kidney were cut and placed inside their labeled cassettes. After routine processing, sections of the embedded tissue blocks were cut at 3µm using rotary microtome and then floated out on labeled glass slides. The cut sections were stained using Haematoxylin and Eosin staining method to demonstrate general tissue structures. Histological examination was done under x10 and x40 objectives. Photomicrographs were taken to demonstrate tissue structures and changes that occurred as a result of the extract administration (Avwioro, 2014).

Data Analysis

Data analysis was performed using GraphPad instat3 version 3.02 (GraphPad Corp., San Diego, USA). Data were presented as mean ± SEM. Statistical comparison between groups were made using one-way analysis of variance (ANOVA) with post hoc Bonferroni Multiple Comparison Test to identify differences in means where appropriate and a $p \leq 0.05$ was taken as statistically significant value

RESULTS

The physical properties of the extract observed after the extraction procedure is shown in table 1. Aqueous extraction of *Senna occidentalis* leaf yielded 10.9% of the powdered leaves; the extract was gummy in texture and has brownish colour.

The phytochemical screening of the *Senna occidentalis* leaf extract is shown in table 2. Carbohydrate, tannins, triterpenoids and proteins were observed to be moderately presence while saponins, steroids, flavonoids, diterpenoids and cardiac glycosides were high in abundance; while alkaloids and anthraquinones are not detected by the screening methods employed.

Table 1:
Physical properties of *Senna occidentalis* leaf extract

Plant Part	Type of Extract	%Yield	Texture	Colour
Leaf	Aqueous extract	10.9%	Gummy	Brown

Table 2:
Phytochemical analysis of *Senna occidentalis* leaf extract

Phytochemical	Test	Observation	Result
Carbohydrate	Molisch test	Dull violet colour	+
Saponins	Frothing test	Frothing	++
Steroids	Libermann-Buchard's test	Reddish + blue-green	++
	Salkowki's test	Cherry red colour	++
Flavonoids	Alkaline reagent test	Colourless	++
	Shinoda's test	Orange-red colour	++
Alkaloids	Meyer's test	No precipitate	-
	Wagner's test	No brown precipitate	-
Tannins	Lead sub-acetate test	Blue-black	+
Diterpenoids	Copper acetate test	Green colour	++
Triterpenoids	Libermann-Buchard's test	Red colour	+
Anthraquinones	Bornfrager's test	No colour change	-
Cardiac glycosides	Keller-Killani	Brown + pale green	++
Protein	Xanthoproteic test	Yellow colour	+

***(+)** indicate presence **(++)** indicate significant presence*
***(-)** indicate not detected*

All the graded doses of the aqueous extract of *Senna occidentalis* administered to the albino rats showed no sign of toxicity or behavioral change. After 24 hour observation, no deaths were recorded in both the first phase and second phase of the procedure as shown in table 3.

The LD_{50} of the aqueous leaf extract of *Senna occidentalis* was found to be above 5000mg/kg.

The renal function test was carried out to determine effect of oral administration of the extract on the kidney parameters of Wistar rats and the result is shown in Table 4. There was no significant effect seen between the values of the sodium, potassium, chloride, bicarbonate, urea and creatinine in the control and test groups as per row.

Table 3:
LD_{50} of aqueous leaf extract of *Senna occidentalis* in Wistar rats

Dose (mg/kg)	Observation	
	First Phase	Second Phase
10	0/3	-
100	0/3	-
1000	0/3	-
1600	-	0/1
2900	-	0/1
5000		0/1

Using the formular by Lorke (1983): $LD_{50} = \sqrt{(D_0 \times D_{100})}$
D_0 *= Highest dose that gave no mortality* D_{100} *= Lowest dose that produced mortality.*

Kidney Histology
The glomeruli were well preserved with abundant presence of Bowman's space as shown in Plate 1A. The proximal convoluted tubules are abundantly presence and well preserved while distal convoluted tubules are significantly presence and preserved as shown in plates 1B to 1E. There was absence of inflammation in the interstitium and distortion in the architecture of both the proximal and distal convoluted tubules as shown in Plates 1B to 1E. Focal sclerosis was seen in some of the animals as shown in 1E.

Table 4:
Effect of oral administration of aqueous leaf extract of *Senna occidentalis* on kidney parameters

Parameter (mmol/L)	Different doses (mg/kg)				
	0	350	700	1500	3000
Sodium	131.75 ±4.7003	129.50 ±1.32	134 ±4.18	132 ±5.64	136.75 ±4.72
Potassium	5.73 ±0.57	6.60 ±0.37	5.90 ±0.53	5.0 ±0.29	5.63 ±0.97
Chloride	95.25 ±2.81	93.0 ±1.29	103.75 ±3.22	101.25 ±4.11	97.5 ±2.40
Bicarbonate	25.25 ±1.25	22.75 ±1.25	21.75 ±0.48	21.25 ±0.95	24.50 ±2.06
Urea	5.55 ±0.36	5.85 ±0.86	5.70 ±0.37	6.63 ±0.20	6.55 ±0.34
Creatinine	0.7 ±0.00	0.7 ±0.00	0.73 ±0.03	0.7 ±0.04	0.7 ±0.00

Data are presented as mean ± SEM, $p>0.05$ (not significant).

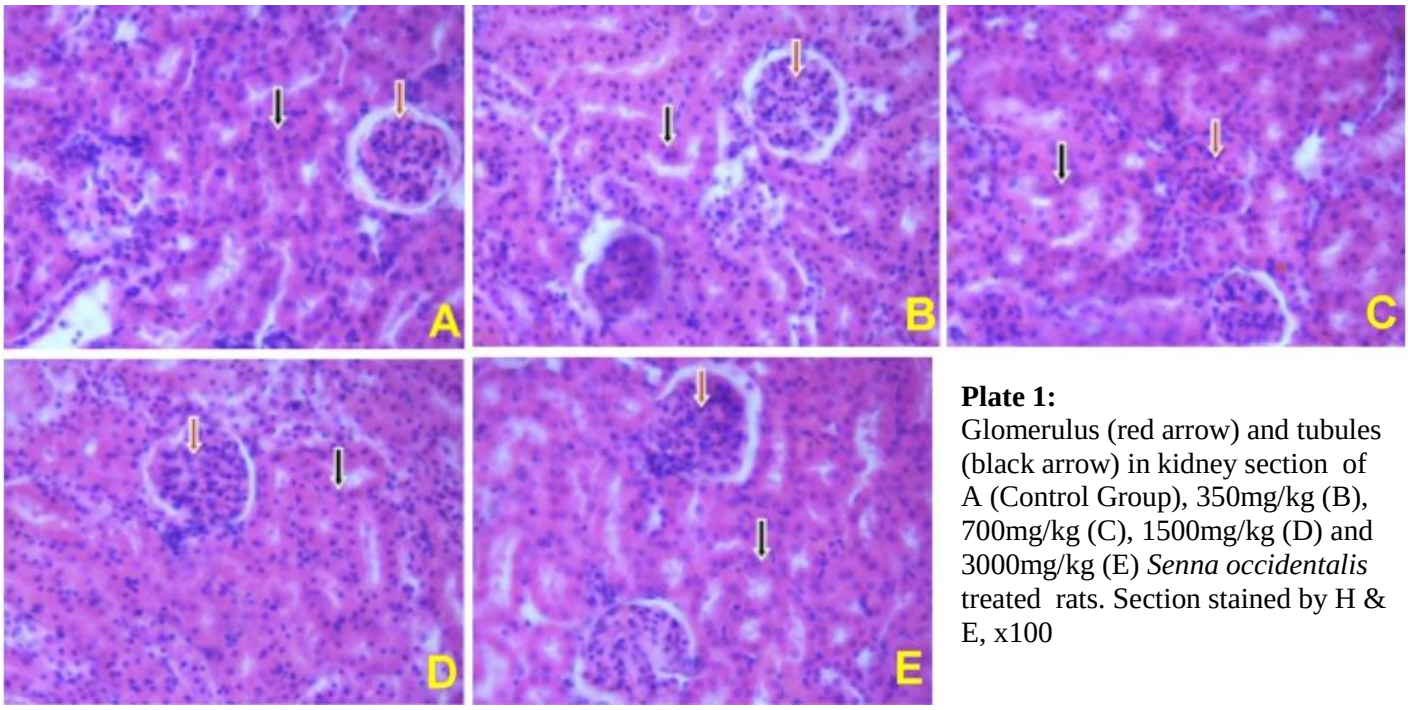

Plate 1:
Glomerulus (red arrow) and tubules (black arrow) in kidney section of A (Control Group), 350mg/kg (B), 700mg/kg (C), 1500mg/kg (D) and 3000mg/kg (E) *Senna occidentalis* treated rats. Section stained by H & E, x100

DISCUSSION

The aqueous extraction method used in this research yielded 10.9% yield of a gummy extract that was brownish in colour. This is consistent with Shafeen *et al.* (2012) that also got 11.3% yield of aqueous leaf extract of *Senna occidentalis*. Phytochemical screening of aqueous leaf extract of *Senna occidentalis* also revealed high presence of saponins, steroids, flavonoids, diterpenoids and cardiac glycosides as well as moderate presence of carbohydrate, tannins, triterpenoids and protein. Alkaloids and anthraquinones were however not detected in the plant extract. This result was in conformity with similar studies conducted by Nuhu and Aliyu (2008) and Shafeen *et al.* (2012). Observations on the phytochemical screening of the extract in this study was however in contrast with Taiwo *et al.* (2013) and Garba *et al.* (2015) that reported presence of anthraquinones in their phytochemical analysis of ethanolic extract of *Senna occidentalis* leaf while Egharevba *et al.* (2010) reported presence of alkaloids in leaf extract of *Senna occidentalis* using hexane, ethylacetate and 98% methanol mixtures. These differences can possibly be attributed to different methods of extraction employed or probably due to environmental variations. However, the presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines (Egharevba *et al.*, 2010).

The LD_{50} of aqueous leaf extract of *Senna occidentalis* was found to be safe up to 5000mg/kg body weight and this result was consistent with Silva *et al.* (2011), Shafeen *et al.* (2012), Tanimu and Wudil (2012), and Vijayalakshmi *et al.* (2013). According to Kennedy *et al.* (1986), substances that present LD_{50} higher than 5.0 g/kg by oral route may be considered practically non-toxic; suggesting that acute toxicity of the *Senna occidentalis* leaf is practically zero. Although this does not predict the lethal dose in humans; it however, provides a basis of suggesting its safety for usage in humans.

Statistically, there was no significant effect seen as on the renal parameters analyzed which was also reported by Silva *et al.* (2011) indicating oral administration of aqueous leaf extract of *Senna occidentalis* did not exert detrimental damage to the kidneys. The histology of the kidneys showed well preserved glomeruli and abundant Bowman's space in both the control and the test groups. Global sclerosis and inflammatory cells are absent in all the animals regardless of the concentration of the extract while only few animals in the treated groups showed mild to moderate focal sclerosis. Proximal and distal convulated tubules are well preserved and there was absence of inflammation in the interstitium. Overall, the architecture of the kidney was well preserved with only few animals showing mild to moderate sclerosis which is not dose-dependent. This finding is consistent with Silva *et al.* (2011) that also reported normal architecture of the kidneys in Wistar rats treated with the extract. The phytochemical screening of the aqueous leaf extract of *Senna occidentalis* found in our locality revealed absence of anthraquinones probably that could be attributed to the non-lethal histopathological effects seen on the kidneys (Tasaka *et al*, 2000).

In this study, oral administration of up to 3000mg/kg body weight of aqueous leaf extract of *Senna occidentalis* did not produce significant effects on kidney of Wistar rats thereby suggesting it non-lethal effect on the kidneys.

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