Research Article

Hematological, Biochemical and Antioxidant Properties of Hydromethanolic Extract of *Senna alata* and *Senna podocarpa* leaves on *Albino Rats*

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ABSTRACT

Senna alata and *Senna podocarpa* are medicinal plants widely used in complementary and alternative medicine in the treatment of hypertension, dermatophytic infections, constipation, convulsion and gonorrhea in southwestern Nigeria. The effect of hydromethanolic leaf extract of *S. alata* and *S. podocarpa* on hematological and biochemical parameters as well as antioxidant liver histopathology were assessed in rodents.

Thirty-five (35) male albino rats divided into seven groups (n = 5) were administered 1 ml daily gavage dose of distilled water, 200-, 600- and 1000- mg kg^{-1} body weight of extract for 63 days. The antioxidant effect was evaluated through the activity of catalase and determination of the concentration of thiobarbituric acid reactive substance (TBARS), a measure of lipid peroxidation in the liver and plasma, while some biochemical and hematologic parameters were also determined.

Both *Senna alata* and *Senna podocarpa* extract significantly ($p<0.05$) elevated the hematocrit level at all doses tested, monocytes were significantly ($p<0.05$) elevated by all doses of *S.alata*. Plasma alanine aminotransferase (ALT) was significantly ($p<0.05$) reduced by 1000 mg kg^{-1} bwt of *S. alata*, whereas alkaline phosphatase (ALP) was significantly ($p<0.05$) elevated by 600 mg kg^{-1} bwt of *S. podocarpa.* Plasma lipid peroxidation was significantly decreased by all doses of extract tested except 600 mg kg^{-1} bwt of *S. alata,* while Plasma catalase activity was not significantly affected. Liver lipid peroxidation was significantly reduced by all tested doses of *S. alata*, while liver catalase activity was significantly decreased by only 200 mg kg^{-1} bwt of *S. alata*. Histopathological analysis of liver showed significant changes in tissue morphology of both *S. alata* and *S. podocarpa* dosed rats in the 600 and 1000 mg kg^{-1} body weight group.

The results from this study suggest a possible role of these extracts in the management/improvement of anemic conditions, while the TBARS reducing capability of the extract suggests some intrinsic antioxidant properties that combat oxidants involved in lipid peroxidation. These results also demonstrate some histopathological effects of high doses of *S. alata* and *S. podocarpa* on the liver under long-term administration conditions

Keywords: Antioxidant, *Senna alata*, *Senna podocarpa,* Catalase. TBARS level, CAM.

INTRODUCTION

Medicinal plants formed the foundation of medicine and treatment of diseases at the very beginning of human civilization (Ajayi *et al.*, 2008), and thousands of plants having medicinal virtues of one kind or the other have been discovered. In developing countries, a good proportion of the population relies heavily on traditional practitioner and medicinal plants to meet primary healthcare needs.

Antioxidants are group of substances which when present at low concentration, in respect to oxidizable substrate, significantly inhibit oxidative process (Aviram, 2000). The results of oxidative process if unattended to leads in a condition termed oxidative stress, signifying an imbalance between oxidant generation and antioxidant response in a biological system. The presence of antioxidants, due to their ability to trap highly reactive oxygen species, has been suggested to reduce the risk of chronic diseases like cancer, sickle cell anemia and heart diseases. The exogenous supply of antioxidant compounds which include whole grain, vegetables, fruits, plants and plant products (Miller *et. al.*, 2000) play an important role as a protecting factor, and primary sources of naturally occurring antioxidants.

There are 33 species of *Senna* growing in Nigeria of which only a few are well known and used for medicinal purposes (Ayo and Amupitan, 2007). S*enna alata* popularly referred to as “asunwon oyibo” and S*enna podocarpa* referred to as “asunwon egba” by the Yoruba tribe in Nigeria, have been widely

employed traditionally in the treatment of several ailments including skin disorders (Ogunkunle and Ladejobi, 2006), ringworm (Idu *et. al*., 2006), convulsion, gonorrhoea (Odugbemi, 2006), pile, heart (Wegwu *et. al*., 2005), abdominal pain and constipation (Sofowora, 1982).

Herbal preparations in most countries are not prescription regulated, and therefore access to these therapeutic agents are unrestricted, leading to their abuse (Stickel *et. al.,* 2005), Liver injury from herbal remedies has ranged from mild elevations of liver enzymes (markers of hepatotoxicity) to fulminated liver failure requiring liver transplantation. Therefore, there is need for scientific investigation of the effects (beneficial and harmful) of herbs on the total well being of man (Odugbemi, 2006). Although these plants are known for their curative properties, there is tendency that they may have toxic effect on major organs of the body most especially the liver, which is the major organ of food and xenobiotics metabolism.

Therefore, the objective of this work is to investigate the hematologic, biochemical and antioxidative effect of hydromethanolic leaf extract of *S. alata* and *S. podocarpa* on adult male albino rats.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Two medicinal plants, *Senna alata* and *Senna podocarpa,* were sourced during the rainy season (July – September, 2007) from Ibadan area of Oyo state in Nigeria, and authenticated by the curator of the Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan. The voucher specimens of *Senna alata* (No.FHI.106929) and *Senna podocarpa* (No.FHI.106995) were deposited at FRIN herbarium. The air dried leaves were grounded and exhaustively extracted in a soxhlet apparatus using 80% methanol as solvent. The crude extract was concentrated under vacuum using a rotary evaporator, freeze dried and stored at 4^oC until use.

Experimental Animals and Treatment

Sexually matured Wistar albino rats, weighing between 94 and 125 g, were obtained from the Animal House of the University of Ibadan, Ibadan, Nigeria. The rats were transferred to the University of Lagos, department of Cell Biology and Genetics' animal House where they were allowed to acclimatize for two weeks. They were maintained in the experimental facility, provided standard feed (rat pellets) for laboratory rodent, and regular tap water *ad libitum*. These rats were randomly distributed into seven groups of 5 rats each.

The rats in group A which served as Normal control did not receive any dose of the extract but was administered 1 ml of distilled water daily. The rats in groups B, C and, D received by gavage, 1 ml daily dose of hydromethanolic leaf extract of *Senna alata* while those in groups E, F, G, received by gavage, 1 ml daily dose of hydromethanolic leaf extract of *Senna podocarpa*, at doses of 200, 600 and 1000 mg kg^{-1} body weight respectively for 63 days. The animals were deprived of food but not water 8 hours prior to administration of the extracts. Animals were weighed before and after the experiment.

At the termination of experiment, all surviving animals were fasted over night and sacrificed after anaesthesia, by decapitation and blood from each animal was collected separately in labelled heparinised / EDTA tubes. The liver was harvested and washed instantly in normal saline. The washed organs were blotted dry with filter paper, weighed and labelled. About 0.2 g of the organs were cut with surgical scissors, kept separately in labelled containers and frozen (-20^0C) for further analysis of antioxidant and biochemical parameters.

Hematology and Biochemical analysis

Blood collected into anticoagulant (lithium heparin or EDTA) tubes were used to determine hematocrit, total, differential and percentage white blood cells count according to Dacie, 1991. Blood collected into anticoagulant tubes were centrifuged at 3000 rpm for 10 minutes at room temperature. The resulting plasma as well as liver homogenate (10 %) was analyzed to determine the activity of some enzymes, e.g Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) using the method of Reitman and Frankel (1957); alkaline phosphatase (ALP) was analyzed by the method of Bessey *et al*. (1946) and total protein (Lowry *et al.,* 1951).

Antioxidant assay

The catalase and TBARS levels of the plasma as well as the liver homogenate were also determined according to the method of Niehaus and Sammuelson, 1999.

RESULTS

Senna alata and *Senna podocarpa* significantly ($P<0.05$) elevated the hematocrit of albino rats, with no significant changes in the white blood cells, platelets and leucocytes populations. *S. podocarpa* did not significantly affect the population of monophils when compared to the control, whereas *S. alata* on the other hand significantly ($P<0.05$) increased

the monophils population. The population of eosinophils was significantly ($P<0.05$) increased compared to control at 1000 mg kg.bwt^{-1} dose of *S. alata*.

Table 1: Effect of 63 days administration of hydromethanolic leaf extract of *Senna alata* and *Senna podocarpa* on hematologic parameters of albino rats

Groups	Dosage (mg kg^{-1})	HEMATOLOGIC PARAMETER					
		Hematocrit (%)	WBC (x10³) (x10^3/mm^3)	Platelets (x10^3/mm^3)	Leucocytes (x10^3/mm^3)	Monophils (x10^3/mm^3)	Eosophils (x10^3/mm^3)
CONTROL (Water)		31.6±0.68	6.04±3.31	40.2±3.1	58.8±3.81	0.4±0.4	0.6±0.6
S. alata	200	41.0±1.03*	7.33±6.23	44.67±1.41	51.0±1.91	2.0±0.0*	2.67±0.61
	600	42.17±0.95*	6.32±5.18	42.17±0.91	54.33±0.99	2.0±0.0*	1.5±0.22
	1000	40.4±1.69*	4.44±1.2	44.0±1.38	51.0±1.18	1.8±0.37*	3.2±0.97*
S. podocarpa	200	39.4±2.04*	6.64±7.54	41.4±1.66	56.8±2.13	0.8±0.37	1.0±0.45
	600	40.0±1.52*	6.24±5.87	34.8±3.37	64.8±3.23	0.0±0.0	0.0±0.0
	1000	38.4±0.70*	5.7±3.42	38.2±3.41	61.4±3.70	0.4±0.40	0.0±0.0

Values are expressed mean ± SEM (n=5); P<0.05 level of significant using ANOVA followed by tukey multiple comparism test.
Asterisk (*) shows that the mean is significantly different (P<0.05) when compared to the control on the same column.

Both plants extract did not significantly affect the activity of plasma ALT, but at 1000 mg kg^{-1} dose of *S. alata,* a significant reduction ($P<0.05$) of AST activity was observed while ALP activity was significantly ($P<0.05$) increased at 600 mg kg^{-1} dose of *S. podocarpa*.

Table 2: Effect of 63 days daily administration of hydromethanolic leaf extract of *Senna alata* and *Senna podocarpa* on activity of some plasma enzymes of albino rats

Groups	Dosage (mg kg^{-1})	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})
Control		24.18±1.05	19.16±0.79	63.29±10.42
Senna alata	200	27.73±1.67	13.61±2.64	73.29±10.00
	600	32.24±7.20	16.41±1.56	78.20±16.78
	1000	22.86±0.80	10.05±1.79*	76.18±10.79
Senna podocarpa	200	21.88±1.23	15.39±1.20	76.36±12.87
	600	40.16±4.23	17.38±0.10	172.00±26.40*
	1000	25.34±4.73	17.38±0.97	90.90±19.33

Values are expressed mean ± SEM (n=5); P<0.05 level of significant using ANOVA followed by tukey multiple comparism test.
Asterisk (*) shows that the mean is significantly different (P<0.05) when compared to the control on the same column.

However *S. alata* and *S. podocarpa* did not significantly alter plasma catalase activity at all doses tested when compared to the control, but 200 mg kg^{-1} dose of *S. alata* significantly reduced ($P<0.05$) the liver catalase activity.
There was a significant ($P<0.001$) reduction in the liver lipid peroxidation compared to the control, at all the tested doses of *S. alata*, while *S. podocarpa* on the other hand did not show significant ($P<0.05$) effect on the liver lipid peroxidation level.
The level of lipid peroxidation in the plasma was significantly ($P<0.05$) reduced over the control by 200 and 1000 mg kg^{-1} of dose of *S. alata*, but *S. podocarpa* at 200 and 600 mg kg^{-1} caused a very high significant ($P<0.001$) decrease in the plasma lipid peroxidation when compared to the control.

Table 3: Antioxidant effect of 63 days daily administration of hydromethanolic leaf extract of *Senna alata* and *Senna podocarpa* on albino rats

Groups	Dosage (mg kg^{-1})	CATALASE		TBARS	
		LIVER	PLASMA	LIVER	PLASMA
CONTROL		0.34±0.04	0.084±0.05	11.35±6.26	5.48±4.68
Senna alata	200	0.07±0.10*	0.297±0.10	5.90±3.24*	2.03±1.08*
	600	0.08±0.09	0.037±0.40	7.28±3.55*	3.51±1.24
	1000	0.05±0.08	0.042±0.25	6.77±7.57*	2.06±1.04*
Senna podocarpa	200	0.33±0.05	0.024±0.02	10.12±1.31	2.71±4.20*
	600	0.40±0.04	0.012±0.00	10.15±0.86	2.25±1.04*
	1000	0.17±0.04	0.098±0.04	10.55±1.04	4.52±1.98

Values are expressed mean ± SEM (n=5); P<0.05 level of significant using ANOVA followed by tukey multiple comparism test. Asterisk (*) shows that the mean is significantly different (P<0.05) when compared to the control on the same column.

DISCUSSION

S. alata and *S. podocarpa* leaves have been used in ethnomedicine to treat various ailments. Therefore it is not surprising that the treated rats showed no changes in their daily physical and behavioural activities. The intake of hydromethanolic leaf extract of *S. alata* and *S. podocarpa* extract did not affect the functions of the bone marrow as reflected by the values of WBC (total and differential) and platelets; neither were they quantitatively nor qualitatively destroyed, consequently leucopoiesis was not affected. It is likely that the extracts may have affected the circulating erythrocytes and possibly hematopoiesis as reflected by changes in packed cell volumes (PCV) and eosinophils. The increase in the PCV values may be taken to be a reflection of the hydration state of the RBCs - culminating in an increased RBC volume - indicative of an impairment of the cellular dehydration pathway, which is mediated by K^+-Cl^- cotransporter (KCC) or Ca^{2+}- activated K^+ channel (Gardos pathway) as suggested by Muzyamba and Gibson (2003).

The changes in aspartate (AST) and alanine (ALT) aminotransferase enzyme levels observed in the rats were not double the value of the control, which is one indicator of biological significance (Rhomberg *et al*., 2007). Both enzymes are not liver specific but are also found in skeletal and cardiac muscle (Thrall, 2004), thus these findings are therefore not considered toxicologically significant.

Quantitative reduction of thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, indicated potential of crude extracts of both plants to inhibit oxidation in lipid system. Thus the findings from this study revealed that the leaf extract of *S.alata* and *S.podocarpa* increased the antioxidant capacity of blood and had an inhibitory effect on the basal level of liver lipid peroxidation, a major consequence of free radical cell damage, which may alter intrinsic membrane properties and also contribute indirectly to other deleterious effects of ischemia/reperfusion, because it increases membrane calcium permeability (Bagchi *et al*., 1997) and enhances phospholipid susceptibility to degradation by phospholipase (Sevanian *et al*., 1981; Weglicki *et al*., 1984). Natural antioxidants, e.g. plants extract, may have also protected erythrocyte membrane from lipid peroxidation induced by oxo-heme oxidants, by terminating the chain reaction or reacting with free radicals, particularly peroxy radicals, which are major propagators of the auto-oxidation chain of fat (Shimada *et al*., 1992). Okpuzor *et al.* (2009), identified Apigenin and Naringin in *S. alata* leaves as the flavonoids which may confer antioxidant potentials. This lends scientific support to the therapeutic use of the plant leaves in traditional medicine.

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