Subchronic toxicity study of *Khaya senegalensis* aqueous extract in rats

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Abstract

This study was carried out to investigate the sub chronic effects of oral administration of different doses: 100mg/kg, 400mg/kg, 1600mg/kg, 6400mg/kg of aqueous extract of *Khaya senegalensis* in wistar rats. The rats were randomly separated to groups "A-G". The group "A" was considered as a non treated control. The other groups B, C, D and E respectively, were test groups and were treated with the extract. The biochemical and haematological parameters were assessed after 21 days of oral administration of the extract. The liver, kidney, pancreas, and heart of various experimental rats were harvested and preserved. The parenchymal tissues were fixed and using a microtome prepared to slides. Histopathological changes were observed using standard methods. There were no significant differences in white blood cell (WBC), Differential leucocytes count (DLC), haemoglobin concentration, packed cell volume (PCV) and Red Blood Cell count (RBC) respectively. There was however a significant ($P < 0.05$) decrease in the serum Alanine amino transferase (ALT) in groups B, C and D and also a significant ($p < 0.05$) decrease in AST levels in group B and E. The blood glucose levels also decreased significantly ($P < 0.05$) in groups concentration in groups C, D, and E. Histopathological examination showed moderate degenerative changes and congestions in the livers and kidneys and pancreas in all treated groups. This gave an insight on the effects of oral administration of an aqueous extract of *Khaya senegalensis* on the haematological and biochemical parameters, with the related tissue pathology, in experimental wistar rats. Conclusion could be made that care should be taken in prolonged administration of high doses of aqueous extract of *Khaya senegalensis* man and animals.

Keywords: *Khaya senegalensis*; Pathology; Biochemical; Hematological; parameters.

Introduction

Khaya senegalensis is a tropical plant belonging to the family *Meliaceae* and grows up to the height of 15-30m and 1m in diameter, it is recognized by its ever green crown of dark shining foliage, pinnate and characteristics round capsules (Muraina *et al.*, 2007). The bark of the plant is very bitter due to the presence of Meliacine, a phytochemical constituent. It is well known in traditional African medicine due to its great medicinal value and its pharmacological potentials to cure many diseases namely: trypanosomosis, syphilis, psychosis, helminthosis, leprosy, emesis, malaria and many other diseases (Gills, 1992; Fall *et al.*, 1998; Alawa *et al.*, 2008; Biobaku *et al.*, 2009).

It is also used in ethno veterinary medicine in the amelioration of cattle suffering from liver fluke infection, ulcers and wounds in camels and donkeys, while in horses, it is used in internal ailment associated with gastrointestinal tract namely; mucoid diarrhoea, colic and bitter tonic for improving appetite and alleviation of anemia (sanni *et al.*, 2005). The photochemical composition were previously investigated to be Scopoleptin, anthraquinones, quinolones, quins, tannins, coparone, limonoids, flavonoids, sterols and reducing sugars, (Ajagbonna *et al.*, 2005; Muraina *et al.*, 2007). The elemental analysis of the plant was previously carried out by Muraina and Mamman, (2004) and was

found to contain iron, copper and lead. Due to the extensive utilization of the plant in ethno-veterinary medicine and in herbal remedies in humans. The objective of the study is to investigate the effects of oral administration of different high graded doses of the aqueous plants crude extract for a relatively prolonged period of 21 days, thus evaluating the sub chronic effects of the plant on haematological, biochemical parameters and some parenchymatous tissue changes.

Materials and Methods

Authentication and Preparation of plant

The plant *K. senegalensis* was obtained from Abeokuta North, Ogun State, Nigeria. The plant was identified and authenticated in the Botany Department of College of Natural and Applied Sciences, University of Agriculture, Abeokuta by a botanist. The plant was dried and extraction of the pulverized bark of the plant was carried out, using an aqueous medium, hot extraction method was used as previously carried out by Ajagbonna and Oyeyili, (2002); Biobaku *et al.*, (2009). The extract was oven dried at temperature of 60-80°C for 24 hours and a dry powder of the crude extract was weighed and dissolved in distilled water to form 10% solution. The percentage yield was also deduced using standard method as previously adopted by Ajagbonna and Oyeyili, (2002); Ajagbonna *et al.*, (2005); Muraina *et al.*, (2007) and was found to be 0.25%.

Experimental Animal and Design

The study was carried out on 90-day old wistar rats, the weight of the rats ranged between 180-200g. The rats were obtained from the animal unit of the University of Agriculture, Abeokuta, Ogun State, Nigeria. The rats were maintained in cages in the laboratory animals unit of the College of Veterinary Medicine, Abeokuta,

University Animal Ethics Committee Guidelines were adhered to throughout the experiment. The animals were fed with laboratory animal feed and were provided with water *ad-libitum*, the animals were allowed to acclimatize to their housing environment for three weeks before the commencement of the experiment.

Twenty five rats were randomly divided in five groups of five animals each. The rats were orally administered with a calculated dose of 100mg/kg, 400mg/kg, 1600mg/kg and 6400mg/kg for group B to E respectively while group A was given distilled water and was considered untreated control. The treated groups were treated for 21 days.

Sample Collection and Analysis

Blood samples were collected in EDTA and lithium heparin bottles, using a 23 gauge needle from the hearts using cardiac puncture from rats euthanized with chloroform. Haematological parameters namely; packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC) and differential leukocytes count (DLC) as previously described by Schalm *et al.*, (1995) were carried out. While haemoglobin and other serum biochemical parameters were analyzed, using commercial kits Randox® made in England and Standard Spectrophotometric methods using a spectronic 21 digital spectrophotometer (Bauch and Lomb, New York) were utilized for the analysis as described by Reitman's and Frankel (1957) and as described by Ducan and Prasse (1992) and Yakubu *et al.*, (2004). The rats were sacrificed at day 21 after administration and postmortem examination was carried out. The rats were sacrificed at day 21 after administration and postmortem examination was carried out. Tissue samples of the liver, kidney,

pancreas and heart were collected from treatment and control groups and were compared and fixed in 10% neutral buffered formalin for histopathological examination.

Statistical Analysis

All data were presented as mean $\pm$ standard error of mean (S.E.M.). Statistical evaluation and significant difference between the means of individual groups were analyzed using Analysis of variance (ANOVA), followed by Duncan test ($P < 0.05$) was regarded as significant.

Results

K. senegalensis administered as high as 6400mg/kg had no significant change on PCV, RBC, Haemoglobin concentration, DLC and WBC respectively as observed in (Table 1 and 2). However, there was a significant ($P < 0.05$) decrease in serum glucose concentration in groups C, D and E as thus, A (105.14 ± 9.64 mg/dl), B (94.83 ± 8.00 mg/dl), C (83.86 ± 4.12 mg/dl), D (61.70 ± 32.34 mg/dl) and E (82.95 ± 4.94 mg/dl), when compared ($p < 0.05$) with the other groups A and B respectively, while the ALT recorded a significant ($p < 0.05$) decrease in groups

B, C, D when compared ($p < 0.05$) with groups A and E as thus: A (56.00 ± 9.42 IU/l), B (35.75 ± 5.60 IU/l), C (35.20 ± 4.03 IU/l), D (35.00 ± 3.21 IU/l), E (47.75 ± 12.94 IU/l). The AST level also recorded a significant ($p < 0.05$) decrease in groups B and E when compared ($p < 0.05$) to other groups A, C, D and its as thus: A (193.80 ± 19.14 IU/l), B (52.00 ± 8.30 IU/l), C (165.80 ± 12.82 IU/l), D (180.00 ± 5.03 IU/l), E (47.75 ± 12.94 IU/l) as shown in table 3.

Histopathology

Histopathological changes showed severe congestion of the central vein and dilatation of the liver sinusoid. (Fig. 1 and 2). The venules and glomerular capillaries of the kidneys were moderately congested in all the treated groups (fig. 3 and 4) with severity increasing with increase in the dose of extract administered. Tubular and glomerular epithelial cells showed mild degenerative changes (fig. 4). Section of the heart muscle showed congestion of coronary veins (fig. 5). The pancreatic interstitium was mildly oedematous (fig. 6)

Discussion

Khaya senegalensis had being proven to be

Table 1: Effect of *Khaya senegalensis* stem bark crude extract on erythrocytes of wistar rats.

Erythrocytes indices	Experimental groups				
	A	B	C	D	E
Haemoglobin (g/dl)	16.44 ± 0.88	13.87 ± 0.54	14.00 ± 0.69	16.17 ± 1.28	13.90 ± 1.14
PCV%	33.20 ± 1.83	29.00 ± 1.35	30.00 ± 1.30	31.00 ± 6.00	30.00 ± 1.70
RBC $\times (10^6/\mu\text{l})$	5.94 ± 0.30	4.95 ± 0.16	5.14 ± 0.18	5.55 ± 1.05	5.30 ± 0.30

* $P < 0.05$ is considered level of significance.

Table 2: Effect of *Khaya senegalensis* stem bark extract on differential leucocyte count of wistar rats.

DLC	Experimental groups				
	A	B	C	D	E
Neutrophils %	35.40±6.088	37.50±2.99	35.80±3.93	26.00±2.00	27.60±1.60
Lymphocytes %	61.60±6.16	61.25±3.326	63.80±3.9	73.50±1.56	71.80±1.56
Eosinophils %	1.80±0.74	2.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Monocytes %	1.00±0.63	1.00±0.00	1.00±0.00	1.00±0.00	1.50±0.50
Basophils %	0.600±0.24	0.00±0.00	0.00±0.0	0.00±0.00	0.00±0.00
WBCx10 ³ /µl	8.00±0.56	7.13±0.43	8.34±0.71	9.95±0.55	9.14±0.69

*P<0.05 is considered level of significance.

promising as an anticoccidial, Maikai *et al.*, (2007) and anti-inflammatory herbal remedy, Agbedahunsi, (2009), other authors documented its antimalarial effects Makinde *et al.*, (1988) while most reaffirm scientifically its anti trypanosomal effect

(Ajagbonna *et al.*, 2005; Muraina *et al.*, 2007; Ibrahim *et al.*, 2008; Biobaku *et al.*, 2009). This study centered mainly on possible effects of the plant when given in high doses on the biochemical, haematological parameters and description of some histopathological changes associated with oral administration in

Table 3: Effect of *Khaya senegalensis* crude extract on serum biochemistry in wistar rats.

Values summarized as Mean ± standard error of mean.

Biochemical indices	A	B	C	D	E
Glu mg/dl	105.14±9.64	94.83±8.00	83.86±4.126*	61.70±32.34*	82.95±4.94*
Prox g/l	56.54±1.74	57.20±1.91	54.20±3.88	60.03±0.167	52.48±3.085
Alb g/l	30.84±0.61	30.65±1.02	29.92±0.62	31.07±0.47	29.90±0.023
Glu g/l	25.70±1.49	26.55±1.45	24.28±1.54	28.97±0.41	22.58±2.48
AST IU/l	193.80±19.14	92.00±8.30*	165.80±12.82	180.00±5.03	47.35±12.94*
ALT IU/l	56.00±9.42	35.75±5.60*	35.20±4.03*	35.00±3.21*	41.75±12.94
ALP IU/l	170.40±11.20	147.60±21.21	166.40±5.78	170.67±15.02	139.50±2.10
Chol mg/dl	22.24±17.02	92.82±4.2750	103.92±86.96	104.70±8.28	99.22±7.84
Trig mg/dl	100.56±7.16	85.85±3.63	89.12±5.10	90.96±5.1	77.23±10.56
Bil. mg/dl	0.70±0.37	0.75±0.029	0.076±0.040	0.80±0.058	0.725±0.025
Crea mg/dl	0.86±0.040	0.93±0.048	0.96±0.024	0.80±0.058	0.85±0.04
Na ⁺ mmol/l	140.60±1.32	139±2.287	139.20±2.45	137.30±1.77	139.50±3.78
K ⁺ mmol/l	3.64±0.32	3.60±0.28	3.68±0.30	3.33±0.15	3.60±0.00

*P<0.05 is considered level of significance.

Fig 1: Congestion of the central vein in liver tissue

Fig 2: Section of the liver showing dilatation of the liver sinusoids

different organs.* The plasma levels of transaminases (AST, ALT, ALP) are biomarkers for hepatotoxicity and these are indicators that give insight on the effects of exogenous agents such as extracts or drugs (Ozeanli *et al.*, 2006). However, the alteration from the average normal

parameters of this biochemical parameters are important since enzymes and blood are in constant circulation after the synthesis and linkage of enzymes with hepatocytes (Janbaz and Gilani, 2000; Yakubu *et al.*, 2005). Alkaline phosphatase, an enzyme found in hepatocytes is a marker enzyme in

Fig.3: Section of the kidney showing congestion of glomerula capillaries

Fig 4: Section of the kidneys with congestion of capillaries

Fig 5: Section of the heart muscles with severe capillaries congestion

liver diseases. Its serum level increases when there is damage to plasma membrane and endoplasmic reticulum of hepatocytes thus; its level is increased during liver diseases (Chauhan and Agarwal, 2006). It is thus, employed to assess the integrity of plasma membranes of hepatocytes (Akanji *et al.*, 1993). Histopathological examination showed congestion of blood vessels and mild degenerative changes, in most of the tissues examined, but did not suggest damage to cell membranes. This gave credence to the finding in this study as regards to ALP had no significant differences ($P < 0.05$) changes in serum concentration, this study shows that there were histological changes in the liver tissue, kidney, pancreas and the heart tissue. The liver tissue however showed marked congestion of the capillaries at high doses. This gave insight of dose dependent tissue

Fig 6: Section of pancreas showing oedema in the pancreatic the parenchyma

degeneration in the liver; this is because the liver is involved in detoxification and metabolism of the plant principles and drugs. These findings are similar to the review of Abatan, (2012).

The result of this study showed decrease (< 0.05) in the plasma level of ALT and AST. This may be attributed to change in the rate of enzyme synthesis and the dynamics of secretion. Decreased level of ALT could have resulted from the response of the hepatocytes to the extract since examination of tissue pathology did not suggest damage to cell membrane. Degenerative changes in cells might have affected the capacity of hepatocytes to synthesize and release the enzyme. This finding is not in concordance with the report of Yakubu *et al.* (2005) where he reported an increased in the level of plasma ALT and AST. The increase in the plasma

level of ALT in the previous study of Yakubu *et al.*, (2005), is likely to the leakage of the enzyme from damaged hepatocytes. The variation could be as a result of difference in the solvent used for extraction in which ethanol was used in the earlier work (Yakubu *et al.*, 2005), whereas water was used in this study. This suggest that water or aqueous medium could be safer as medium of extraction than ethanol, since ethanol could predispose to hepatotoxicity. The geographical location where the plants were obtained might also play an important role in determining the concentrations of the phytochemical constituents of the extract and consequently influencing its toxic potentials. Geographical location of a plant is known to affect the phytochemical composition of the plant (Sofowora, 1993). The decrease in the plasma glucose could be due to the fact that *Khaya senegalensis* given subchronically induced a relative hypoglycaemic effect due to the presence of the active constituent meliacine, which is a bitter principle that might have altered gastrointestinal hormones and enzymes secretion when absorbed in the gastrointestinal tract. There is an evidence of bitters increasing gastrointestinal secretion (Aliu, 2007). This may bring about increased hepatic activity and most probably increased glycolytic activity due to the frequency of molecules of gastrointestinal secretions and enzymes acting on the glucose and other substrata of other sugar molecules bringing about metabolic utilization, energy or assimilation as related by Dhanotiya, (2006) Thereby bringing about increased metabolic rate and hypoglycaemic effect due to increased utilization of glucose. The *K. senegalensis* extract did not induce any haematological changes in rats when administered orally. This suggests that the

extract had no effect on the haematological values. More so, *K senegalensis* has been reported to have antianaemic effect in pheynylhydrazine induced anaemia in rats (Sanni *et al.* 2006). The plant was claimed to have cytoprotective property which helped the erythrocytes from being predisposed to haemolysis. It was previously advocated that it possesses antioxidative property conferred to it by its active principles including flavonoids and other reducing sugar. In this study however, the *K. senegalensis* extract did not induce erythropoiesis, this could infer non significant changes in the erythrocytic values. Thus, the administration of high doses of *K. senegalensis* did not show significant clinical signs of toxicity. However mild variation in biochemical parameters suggests some effects of oral administration of *K senegalensis*. The changes in the biochemical parameters may be due to the slight degenerative changes in the pancreas which would have contributed to the slight biochemical changes indirectly, since it is an endocrine and an exocrine gland. The adverse effect of the plant on the hepatocytes and liver tissues may be speculated to be reversed by regeneration of the tissue, if they exposure by administration of the extract is withdrawn. Care however, should be taken when the plant is administered to diabetic subjects and patients with history of hypoglycaemia and in congestive heart failure because the histological changes showed congestion of the capillaries of the musculature of the heart at relatively high doses. There is need for further research to ascertain the safety of the therapeutic use of *K senegalensis* using more sensitive biochemical molecular technique and cardiac troponin I to reaffirm its safety in man and animal.

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