Liver and Kidney Functional Indices of Pregnant Rats Following the Administration of the Crude Alkaloids from *Senna alata* (Linn. Roxb) Leaves

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ABSTRACT

Background: Alkaloids from *Senna alata* leaves implicated as the active constituents of abortifacient are yet to be investigated for their effects on the normal functioning of the maternal liver and kidney. Therefore, the effects of crude alkaloids on some biochemical indices of kidney and liver damage were investigated in pregnant rats.

Methods: Pregnant rats were randomized into 4 groups: A (control), B, C, and D and were orally administered 0.5 ml of distilled water, 250, 500 and 1000 mg/kg body weight of the alkaloids respectively once daily on days 10-18 post coitum.

Results: Thin-layer chromatographic separation gave five spots with R_f values of 0.28, 0.33, 0.39, 0.47, and 0.55 that produced creamy precipitate and reddish-brown colour, respectively, with Mayer's and Wagner's reagents. Quantitative determination gave 0.30 g which corresponded to a percentage yield of 1.50 % of the alkaloids. The decreases in the activities of alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), aspartate (AST) and alanine transaminases in the liver and kidney of the animals by the alkaloids were accompanied by corresponding increases in the serum enzymes. The alkaloids reduced liver- and kidney-body weight ratios, serum globulin, urea, uric acid, and phosphate ions while the serum concentrations of albumin, bilirubin, creatinine, potassium ions, AST/ALT ratio, blood urea nitrogen: creatinine increased. The levels of sodium, calcium, and chloride ions did not change significantly ($P>0.05$).

Conclusion: Overall, the alkaloid at doses of 250-1000 mg/kg body weight produced permeability changes in the plasma membrane of the organs and adversely affected the normal secretory, synthetic, and excretory functions of these organs.

Keywords: Alkaloids, function indices, leguminosae, pregnant rats, safety, *Senna alata.*

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INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value known as phytochemicals. While phytoconstituents, such as alkaloids, flavonoids, tannins, saponins etc. are responsible for the diverse pharmacological activities like anticancer, aphrodisiac, antimalarial, antidiabetic and abortifacient, they also serve as "lead compounds" or templates for the rational development of drugs. These compounds have also been reported to be toxic in animals when ingested at certain concentrations (1).

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Some of these damages include reproductive dysfunction, such as antifertility and spermatotoxicity, and organ dysfunction. Therefore, it has become imperative to assess the safety of these phytochemicals obtained from medicinal herbs like *Senna alata*.

Senna alata Linn. Roxb (Leguminosae), also known as Craw-Craw plant or Ringworm plant (English), asunwon oyinbo (Yoruba-Western Nigeria), Nelkhi (Igbo-Eastern Nigeria), Filisko or Hansti (Hausa- Northern Nigeria), is indigenous to Africa. It is an annual, erect, tropical herb of 0.15 m high. The leathery, large, compound, bilateral leaves fold together at night. The fruit is a pod, while the seeds are small and square in shape. Several uses have been ascribed to this plant in the traditional medicines of some African countries. These include the management of hepatitis, skin diseases, jaundice, gastroenteritis, ringworm, eczema, and diarrhoea (2). Information from the ethnobotanical survey conducted on some localities in Nigeria has revealed that the plant is also claimed to be used as an abortifacient for 'washing the uterus' (3).

Preliminary phytochemical screening has shown that the aqueous leaf extract of *S. alata* consists of saponins (1.22%), flavonoids (1.06%), cardiac glycosides (0.20%), phenolics (0.44%), alkaloids (0.52%), cardenolides, and dienolides (0.18%) (3). The antifungal, antibacterial, and antioxidant activities of the plant have also been supported with scientific data (4-6). Furthermore, Yakubu *et al*, (2010) (3) provided scientific basis to the acclaimed use of the aqueous leaf extract of *S. alata* as a pregnancy terminator (abortifacient), and attributed this to any of alkaloids, flavonoids, and saponins which might have acted either singly or in combination (3). In addition, the alkaloids derived from *S. alata* leaves at the doses of 250, 500, and 1000 mg/kg body weight administered once daily from days 10 until day 18 post-coitum have been reported to exhibit abortifacient activity in a manner similar to the aqueous leaf extract of the plant. Apart from the report on the effect of the alkaloid on the fetal and maternal outcomes in pregnant rats, there has not been any other report in the open scientific literature that has addressed the effect of the active constituents of abortifacient on the normal functioning of the liver and kidneys of pregnant rats.

Therefore, this study was done to evaluate the toxicological implications of the crude alkaloids derived from *Senna alata* leaves at 250, 500, and 1000 mg/kg body weight doses, administered once daily from day 10 until day 18 post-coitum, on the function indices of the liver and kidneys of pregnant rats.

MATERIALS AND METHODS

Plant material and authentication

Senna alata leaves were obtained from a herbseller at a market (Oja-tuntun) in Ilorin, Kwara State, Nigeria. The plant was authenticated at the Herbarium Unit of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, and allocated a voucher number of FHI 10845.

Chemicals and reagents

The assay kits for urea, phosphate and calcium ions were the product of AGAPPE Diagnostic Ltd., Agappe Hills, India, while the chloride ion assay kit was from DIALAB Production and Vertrieb Von Chemisch, Austria. The assay kits for AST, ALT albumin, bilirubin, globulin, uric acid, creatinine, sodium and potassium ions were the products of Randox Laboratories, Ltd, Co. Antrim, United Kingdom. The L-γ-glutamyl-p-nitroanilide and glycylglycine were products of Sigma Aldrich, Buchs, Canada and EMD Bioscience, Inc, Darmstadt, Germany, respectively while Para-nitrophenyl phosphate was a product of Sigma-Aldrich Inc., St. Louis, USA. All other reagents used were of analytical grade and were

prepared in the laboratory using glass-distilled water. Thin layer chromatographic (TLC) plates and silica gel were products of Merck (Darmstadt, Germany).

Animals

Male and female Wistar rats (*Rattus norvegicus*) weighing 178.91±3.07 and 143.99 ± 1.21 g, respectively, were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals which were housed individually in plastic cages placed in a well-ventilated room (temperature: 22 ± 3ºC; photoperiod: 12 hours natural light and 12 hours darkness; humidity: 45-50%) were provided with unrestricted access to rat pellets (Bendel Feeds and Flour Mills, Ewu, Nigeria) and water. The rats were handled according to the guidelines of the European Convention for the protection of vertebrate animals and other scientific purposes- ETS-123 (2005) (7).

Extraction of alkaloid

The leaves of *Senna alata* were oven-dried at 40^0C for 48 hours and pulverised with a Mikachi Blender (MK-1830, China). The alkaloids were extracted from the powder by adopting the procedure described by Manske (1965) (8). In short, 500 g of the powder was extracted in 1.2 L of hexane for 72 hours and filtered with Whatmann filter paper No 1. The filtrate (hexane extract) containing fats, oils, terpenes, and waxes were discarded and the residue which was then soaked for a week in 1.2 L of methanol was filtered again. The filtrate was evaporated using a rotavapor (R110, Gallenkamp, England, UK); the recovered methanol was poured into the residue again and left undisturbed for 2 hours.

This was repeated one more time to extract most of the alkaloids from the residue. The resulting filtrates (three) were combined together and concentrated further on a water bath until most of the methanol had evaporated. The methanolic, green slime (90 g), was kept for the next step. The residue (post methanol) was treated with 1 M HCl to extract the remaining alkaloids after which the mixture was filtered again. The residue was discarded, while the filtrate was added to the methanolic green slime; more diluted HCl was added to the solution. This acidic solution was then basified with 5M NaOH and continuously stirred until the precipitate became cloudy.

A known volume (500 ml) of chloroform was then added to the solution, shaken, placed in a separating funnel, and allowed to separate into two layers; 200 ml of 1M NaCl was added to facilitate the separation by breaking down the emulsion formed. This was repeated three times to extract most of the alkaloids from the aqueous (upper) layer into the organic (lower) layer. The organic layer was, then, carefully collected into a conical flask after and a solution of 150 ml of 1M NaCl and 150 ml of 5M NaOH were added. The resulting solution was put in a separating funnel after which the aqueous layer was discarded. The remaining organic layer was then evaporated using water bath and the resulting brownish-black slurry (18 g) which was moderately pure alkaloids that corresponded to a yield of 3.60% was refrigerated at -4ºC and used for the subsequent experiments.

Thin layer chromatographic study of the alkaloids

Thin layer chromatography (TLC) plates were prepared according to the procedure described by Singh and Sahu (2005) (9). The homogenous slurry which was prepared by mixing the silica gel G in double distilled water (1:3, w/v) with constant stirring using a magnetic stirrer was applied to the glass plates (gel $60F_{254}$) with the aid of an applicator to give a layer with a thickness of 0.25 mm. The plates were later air-dried at room temperature (28 ± 2ºC) and further activated at 100 ± 2ºC for 1 h. The activated plates were

stored in desiccators over anhydrous silica gel.

About 10 µl of the test solution (extract) was spotted on the thin layer plate with the help of a micropipette. The plates were developed in the chosen solvent system of chloroform and methanol (10:2) in which 0.01 gm/ml of butylated hydroxyl toluene and butylated hydroxyl anisole were added to prevent oxidation (which may lead to increase in the number of bands with time) (10).

The plates were thereafter withdrawn from the TLC chamber, dried at room temperature and exposed to iodine vapour. Chromatograms were visualized under ultra violet light (UV 254 nm). The alkaloids were confirmed on the thin layer plates with Mayer's and Wagner's reagents. The relative to solvent front (R_f) of the spots were also computed.

Animal grouping and extract administration

The female rats were paired overnight with male rats in the ratio of 1:1 in the aluminium floor cages that allowed free access to food and clean water. The day when a vaginal plug or spermatozoa (detected with the aid of light microscope) appeared in the vaginal smear of the animal was considered as day zero of the pregnancy. Twenty four pregnant rats were completely randomized into four groups (A, B, C and D) consisting of six animals each. Group A (controls), received orally 0.5 ml of distilled water with the aid of an oropharyngeal cannula while animals in Groups B, C, and D received the same volume of the alkaloid corresponding to 250, 500 and 1000 mg/kg body weight, respectively. The doses previously used for the evaluation of abortifacient activity of the aqueous leaf extract of *S. alata* in pregnant rats were also adopted in the present study (3).

The extract and distilled water were administered once daily from day 10 until day 18 of pregnancy (period of organogenesis in Wistar rats) (11). The animals were sacrificed 24 hours after their last dose.

Preparation of serum and tissues supernatants

Under ether anaesthesia, the neck area of the rats was quickly shaved to expose the internal jugular veins. After being slightly displaced (to prevent blood contamination by interstitial fluid), the veins were sharply cut with sterile scapel blade and 5 ml of the blood was collected into clean and dried sample bottles.The blood was allowed to clot for 10 min at room temperature and then centrifuged at 894 × g for 15 minutes using uniscope laboratory centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 hours of preparation for various biochemical assays. The liver and kidneys were then removed from the animals, freed of surrounding tissues and fats, blotted in tissue paper, and weighed for the computation of the organ-body weight ratio. The organs were separately homogenized in 0.25M sucrose solution using Teflon Homogenizer. The homogenates were further centrifuged at 1398 × g for 15 min to obtain the supernatants, which were stored frozen in specimen bottles and used within 24 hours of preparation.

Determination of biochemical parameters

The activities of the enzymes were determined according to the procedures described for alkaline phosphatase (ALP), AST, ALT, and gamma glutamyl transferase (GGT) (12-14). Other parameters evaluated included urea, creatinine, uric acid, bilirubin, albumin, globulin, phosphate, chloride, sodium, potassium, and calcium ions (15-21). The blood urea nitrogen (BUN): creatinine ratio was computed as the ratio of serum urea to creatinine.

Statistical analysis

The computation of the mean and statistical analysis was done using SPSS software (Version 16.0, Chicago, USA). Data which were expressed as the mean ± SEM for group of six animals were statistically analyzed with one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). For all the tests, results with *P* values < 0.05 were taken to imply statistical significance.

RESULTS

The extract from *Senna alata* leaves was positive to both Mayer's and Wagner's reagents as evidenced by creamy precipitate and reddish-brown colour, respectively. Quantitative determination gave 0.30 g which corresponded to a percentage yield of 1.50 % of alkaloids.

The computed liver- and kidney-body weight ratios of the pregnant animals were reduced by all the doses of the alkaloids (Figure 1).

The alkaloids at the various doses of 250, 500 and 1000 mg/kg body weight significantly (P<0.05) decreased the activities of ALP and GGT in both the liver and kidney of the animals (Table 1). These decreases, which were not dose-dependent, were, however, accompanied by corresponding increases in the serum enzymes (Tables 1 and 2).

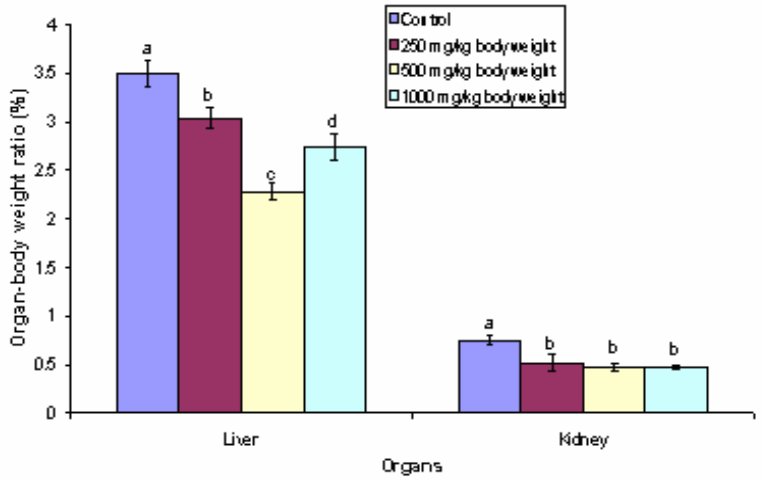

Figure1: Liver and kidney-body weight ratio of pregnant rats administered 10-18 days post-ocitum with the alkaloid from senna alata leaves

Table 1. Alkaline phosphatase activity (nM/min/mg protein) of the liver, kidney, and serum of pregnant rats administered the alkaloids isolated from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Organs/Serum	**Control**	**250**	**500**	**1000**
Liver	2.61 ± 0.03^{a}	1.58 ± 0.03^{b}	1.83 ± 0.00^{c}	1.05 ± 0.08^{d}
Kidney	9.72 ± 0.19^{a}	7.53 ± 0.12^{b}	5.32 ± 0.06^{c}	7.26 ± 0.22^{b}
Serum	0.44 ± 0.00^{a}	0.60 ± 0.03^{b}	0.57 ± 0.03^{b}	0.55 ± 0.05^{b}

Values are means ± SEM of 6 determinations;
Test values carrying superscripts different from the control across the row for the organs and serum are significantly different (P<0.05).

Table 2. Gamma glutamyl transferase activity (IU/mg protein) of the liver, kidney and serum of pregnant rats administered the alkaloids derived from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Organs/Serum	**Control**	**250**	**500**	**1000**
Liver	50.94 ± 1.32^{a}	18.56 ± 0.73^{b}	14.00 ± 0.45^{c}	8.09 ± 0.55^{d}
Kidney	111.00 ± 5.79^{a}	69.56 ± 9.00^{b}	79.91 ± 7.76^{b}	65.97 ± 11.71^{b}
Serum	24.48 ± 0.51^{a}	34.12 ± 2.50^{b}	36.14 ± 0.73^{b}	52.61 ± 1.24^{c}

Values are means ± SEM of 6 determinations.
Test values carrying superscripts different from the control across the row for the organs and serum are significantly different ($P<0.05$).

The activities of both ALT and AST decreased significantly in the kidney and liver of the animals following the administration of the alkaloids isolated from *S. alata* leaves. These decreases were not dose dependent. Furthermore, the alkaloids significantly increased the activities of ALT and AST in the serum of the animals (Tables 3, 4). The alkaloids also increased the computed AST/ALT ratios of the liver, kidney, and serum (Table 5).

There was significant increase in the serum concentration of both the albumin and bilirubin of the pregnant animals whereas the serum globulin content decreased significantly ($P<0.05$) (Table 6).

Similarly, the alkaloids also reduced the concentrations of urea, uric acid and phosphate ions in the serum of the animals, whereas the levels of creatinine, potassium ions and computed blood urea nitrogen: creatinine increased significantly. Furthermore, the alkaloids did not significantly affect ($P>0.05$) the levels of sodium, calcium, and chloride ions in the serum of the animals (Table 7).

Table 3. Aspartate transaminase activity (U/L) of the liver, kidney and serum of pregnant rats administered the alkaloids derived from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Organs/Serum	**Control**	**250**	**500**	**1000**
Liver	170.08 ± 16.43^{a}	128.11 ± 16.43^{b}	120.75 ± 26.43^{b}	120.03 ± 12.43^{b}
Kidney	945.00 ± 49.30^{a}	206.83 ± 27.93^{b}	312.00 ± 19.71^{c}	504.33 ± 32.86^{d}
Serum	52.50 ± 4.82^{a}	72.50 ± 8.21^{b}	84.50 ± 6.19^{c}	84.50 ± 5.45^{c}

Values are means ± SEM of 6 determinations.
Test values carrying superscripts different from the control across the row for the organs and serum are significantly different ($P<0.05$).

Table 4. Alanine transaminase activity (U/L) of the liver, kidney and serum of pregnant rats administered the alkaloids isolated from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Organs/Serum	**Control**	**250**	**500**	**1000**
Liver	216.44 ± 15.74^{a}	177.30 ± 9.71^{b}	180.00 ± 3.29^{c}	158.11 ± 5.15^{d}
Kidney	564.10 ± 10.22^{a}	333.00 ± 9.57^{b}	339.15 ± 11.43^{b}	425.30 ± 10.28^{c}
Serum	53.50 ± 10.95^{a}	92.17 ± 2.73^{b}	116.00 ± 7.89^{c}	133.00 ± 3.29^{b}

Values are means ± SEM of 6 determinations.
Test values carrying superscripts different from the control across the row for the organs and serum are significantly different ($P<0.05$).

Table 5. AST: ALT ratio of the liver, kidney and serum of pregnant rats administered the alkaloids isolated from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Organs/Serum	**Control**	**250**	**500**	**1000**
Liver	1:1.27	1:1.39	1:1.49	1:1.32
Kidney	1:0.60	1:1.60	1:1.09	1:0.84
Serum	1:1.02	1:1.27	1:1.37	1:1.57

Table 6. Some liver function indices of pregnant rats administered the alkaloids isolated from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Parameters	**Control**	**250**	**500**	**1000**
Serum albumin (g/L)	21.94 ± 3.64^{a}	29.55 ± 3.57^{b}	28.53 ± 2.59^{b}	34.34 ± 2.15^{c}
Serum bilirubin (mg/dL)	0.80 ± 0.03^{a}	1.07 ± 0.04^{b}	1.09 ± 0.05^{b}	1.51 ± 0.00^{c}
Serum globulin (g/L)	11.90 ± 0.16^{a}	6.60 ± 0.16^{b}	5.65 ± 0.08^{c}	9.05 ± 0.82^{d}

Values are means ± SEM of 6 determinations.
Test values carrying superscripts different from the control across the row for each serum parameter are significantly different ($P<0.05$).

Table 7. Serum kidney function indices of pregnant rats administered the alkaloids isolated from *Senna alata* leaves on days 10-18 post-coitum

	Extract (mg/kg body weight)			
Parameters	**Control**	**250**	**500**	**1000**
Urea (g/L)	26.16 ± 3.29^{a}	19.25 ± 0.82^{b}	21.75 ± 0.27^{c}	21.83 ± 0.80^{c}
Creatinine (µmol/L)	420.28 ± 0.00^{a}	464.63 ± 0.00^{b}	464.62 ± 4.23^{b}	475.69 ± 2.11^{c}
Blood urea nitrogen : creatinine ratio	1:16	1:24	1:21	1:22
Uric acid (mmol/L)	2.54 ± 0.31^{a}	1.77 ± 0.06^{b}	1.67 ± 0.01^{b}	1.13 ± 0.02^{c}
Sodium ion (mmol/L)	215.00 ± 8.94^{a}	213.67 ± 6.83^{a}	213.33 ± 6.83^{a}	210.00 ± 9.49^{a}
Potassium ion (mmol/L)	4.00 ± 0.09^{a}	5.30 ± 0.51^{b}	5.00 ± 0.44^{b}	5.10 ± 0.68^{b}
Calcium ion (mmol/L)	4.60 ± 0.33^{a}	4.55 ± 0.82^{a}	$4. 57 \pm 0.13^{a}$	4.75 ± 0.24^{a}
Chloride ion (mmol/L)	445.00 ± 63.62^{a}	444.67 ± 55.91^{a}	442.50 ± 48.55^{a}	443.33 ± 58.80^{a}
Phosphate ion (mmol/L)	11.75 ± 0.00^{a}	3.60 ± 0.00^{b}	2.82 ± 0.13^{c}	2.95 ± 0.38^{c}

Values are means ± SEM of 6 determinations.
Test values carrying superscripts different from the control across the row for each serum parameters are significantly different ($P<0.05$).

DISCUSSION

The creamy precipitate and reddish brown colour produced with Mayer's and Wagner's reagents respectively further confirmed that the fraction used in the present study consisted of alkaloids, in this instance, from a low-yielding plant.

Secondary plant metabolites, such as alkaloids, perform several functions in plants and may exhibit different biochemical and pharmaceutical actions in animals when ingested, and in micro-organisms even upon exposure. Such actions range from cell toxicity to cell protective effects (22).

The decreases in both the computed liver- and kidney- body weight ratios of the pregnant animals in the present study may suggest atrophy as reflected by the decreases in the enzymes and other biomolecules following the administration of the alkaloids.

The liver and kidney contain numerous enzymes some of which are also present in the serum in very low concentrations. These enzymes which play specific roles in the normal functioning of the organs have no known functions in the serum other than corroborating or indicating damage to the hepatocytes and nephrons. Furthermore, the alterations in the secretory, synthetic, and excretory biomolecules of the liver, such as albumin, bilirubin, globulin as well as the creatinine, urea, uric acid, and serum electrolytes of the kidney can also be used as indicators of impaired organ function or organ dysfunction.

ALP is a ubiquitous enzyme localized within the plasma membrane and can be used to assess the integrity of the plasma membrane, whereas GGT, a microsomal enzyme present in the hepatocytes, renal tubules, pancreas, and intestine is also located within the cell membrane where they transport peptides into the cell and across the cell membrane (23). The decreases in both the activities of ALP and GGT in the liver and kidney as well as the corresponding elevation of the serum enzymes may be related to structural damage to the liver and kidney. Although, no histological examination was carried out in the present study, it is possible that the ordered lipid bilayer of the plasma membrane might have been distorted, leading to the elevated serum enzymes. Furthermore, since GGT is also a marker of oxidative stress, the trend of the enzyme in the present study could also be a reflection of oxidative stress suggesting that the polyunsaturated fatty acids in the membrane might have been oxidized by the alkaloids or its metabolites and, consequently, the damage to the membrane structure (24). Such 'injury' will not only affect metabolic processes that are dependent on the enzymes but may also inhibit the normal functioning of the organs.

AST and ALT are two of the most reliable markers of hepatocellular injury or necrosis whose levels are elevated in a variety of hepatic disorders or dysfunction. Of these transaminases, ALT is more specific for hepatic injury because it is present mainly in the cytosol of the liver and low concentrations elsewhere (25). The pattern of activities of ALT and AST in the liver, kidney, and serum of the animals by the alkaloids further supports the hepatocellular damage proposed earlier in this study. Since an increase in AST:ALT ratio is a reflection of impairment in the normal functions of the organs, it is possible that the liver and kidney functions might have been impaired as reflected by the increased AST/ALT ratio in the present study (26). This further implies that such impairment might have arisen from structural damage to the liver and kidney as evidenced by the leakage of enzymes from the tissue to the serum. In general, liver damage can be divided into direct destruction of hepatocytes or impairment of bile flow. In the early stage of liver damage, cytoplasmic enzymes in hepatocytes may leak from cells into blood whose membrane permeability has been increased (27). Therefore, the damage to the liver might be due to direct destruction of the hepatocytes as revealed by the leakage of the cytoplasmic enzymes (AST and ALT) into the extracellular fluid, the serum in this instance. The findings on AST and ALT in the present study contrast that of Atere and Ajao (2009) following

the administration of crude alkaloid from *Cnestis ferruginea* to male Wistar rats (28). These differences may be due to factors such as the doses used, sex and the state (pregnancy) of the animals.

The alkaloids also adversely affected the synthetic and excretory functions of the liver as evidenced by the alterations in the levels of albumin, bilirubin, and globulin. The elevated serum albumin coupled with reduction in the level of globulin, a reliable indicator of the synthetic function of the liver, suggests adverse effects on the normal functioning of the organ. Furthermore, the increased concentration of bilirubin may be due to overproduction of bilirubin; impaired uptake conjugation or excretion of bilirubin; or regurgitation of conjugated or unconjugated bilirubin from damaged liver cells or bile ducts (29). It also indicated derangement of the liver functions. It is possible that the alkaloids caused increased functional activity in the case of albumin, whereas it impaired the normal synthesis or increased the catabolism of globulin. The normal excretory function was also compromised by the alkaloids leading to elevated bilirubin. All these may be the result of hepatocellular damage by the alkaloids. The findings in the present study, with respect to albumin and bilirubin, agree with the report by Atere and Ajao (2009) following the administration of crude alkaloid from *C. ferruginea* to male rats (28).

Alteration in the serum concentrations of creatinine, urea, uric acid, and electrolytes such as Na^+, Ca^{2+}, K^+, PO_4^{2-} and Cl^- could be employed to indicate renal dysfunction at both the tubular and glomerular levels. Creatinine is formed by non-enzymatic breakdown of creatinine, and changes in the serum concentration could be the result of renal blood flow, renal function and or urine flow (30, 31). The elevated serum concentrations of creatinine, a reliable indicator of impaired glomerular filtration, as well as the enhanced potassium ion, which implies dysfunction at the tubular levels, clearly support impaired renal function. Furthermore, the reduction in the levels of urea, uric acid, also suggests enhanced glomerular filtration rate, whereas the decrease in phosphate ions is an indication of tubular dysfunction. It is not immediately clear why the alkaloids altered the levels of some electrolytes and sparred some, but it may not be unconnected with selective disturbance of the normal renal functioning of the kidney in the pregnant animals. It is clear that urea is synthesized in the liver and excreted by the kidney and to unravel whether it is the liver or kidney that has been affected, the blood urea nitrogen: creatinine was computed. The increase in the computed BUN: creatinine in the present study suggests that the reduction in the serum urea content of the animal is a consequence of renal dysfunction.

From the existing evidence in this study, it is possible to propose the mechanism of action of toxicity of the phytochemical as follows: 1. The alkaloids disrupted the ordered lipid bilayer of the membrane either by oxidising the polyunsaturated fatty acids (PUFA) or by creating pores on the plasma membrane of the hepatocytes and nephrons. 2. There was permeability changes and increased membrane fluidity. 3. These resulted in leakage of the cytoplasmic enzymes from the tissues to the serum and alterations in the levels of secretory, synthetic and excretory biomolecules of the organs. 4. The deranged structure, leakage of the enzymes, and alterations in the levels of the biomolecules may account for the alkaloid-induced toxicity in the animals and, consequently, the dysfunction of the organs in the present study.

CONCLUSION

The present study concludes that the alkaloids from *Senna alata* leaves at the doses of 250, 500 and 1000 mg/kg body weight adversely affected the liver and kidneys of the pregnant animals. The effect

might be due to structural damage to the plasma membrane of the hepatocytes and nephrons which resulted in leakage of enzymes from the tissues to the serum as well as the alterations in the synthetic, secretory, and excretory functions of the organs. The alkaloids have therefore caused disturbances in the normal hepatic and renal functioning of the pregnant rats.

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