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Evaluation of the Effect of *Aloe barbadensis* Root Extract on the Functional Indices of the Liver and Kidney of Male Wistar Rats

J.O. Erhabor* and M. Idu

Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria.

*Corresponding author; Email: joseph.erhabor@uniben.edu Tel: +234 (0) 807 797 9390

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ABSTRACT: The effect of the ethanol extract of *Aloe barbadensis* root on selected serum biochemical parameters was investigated. Sixty male Wistar rats were randomly selected and administered 100, 200 and 400 mg/kg of the extract and 0.2ml/kg of distilled water (control) for 14 days. The results of the liver marker enzymes revealed that there were non-significant changes ($p>0.05$) in alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels on days 1, 7 and 14 of treatment. The concentrations of other liver biomarkers (total bilirubin, direct bilirubin, albumin and total proteins) were also not significantly ($p>0.05$) altered. Selectively, on day 7, the concentration of total protein was significantly ($p<0.05$) different at all treated doses from the control. The extract had no significant ($p>0.05$) effect generally on the kidney's functional indices. At 100 and 200 mg/kg, selective alterations in some renal biomarkers (creatinine, urea, phosphorous and chloride) on different days of treatment were noticed. The results indicate that within the doses used in the study, the ethanol extract of *A. barbadensis* root is relatively non-toxic with no significant localized toxicity.

Keywords: *Aloe barbadensis*, Toxicity, Functional indices, Kidney, Liver

Introduction

Generally, Traditional medicine practitioners and other users of medicinal plants believe that herbs are safe. This belief results from the plants' natural origin (Afolayan and Yakubu, 2009). Some of these herbs have chemical substances capable of producing damaging or detrimental effects on man and animals. This harmful effect can be on the cell (cytotoxicity), liver (hepatotoxicity), and kidney (nephrotoxicity). Nevertheless, these injuries' occurrences depend on the number of chemicals absorbed (Betram, 1998). It has been observed that most of these herbs have been negated by adequate toxicity data to ascertain their safety and a lack of dose regime (Pousset, 1988). It is pertinent that appropriate scientific investigation of any medicinal plant's beneficial and harmful effects be done (Idu *et al*., 2006). The toxic effect of a drug in man has been reported akin to that of some animals. Therefore animal models are used in toxicological studies (Range *et al*., 1995). Validating the toxicity of any chemical substance or medicinal plant has helped determine the upper limits of effective therapy (Sofowora, 1993). Of the several medicinal plants with a myriad of traditional uses is *Aloe barbadensis.*

Aloe barbadensis Mill. commonly called *Aloe vera*, belongs to the family-Xanthorrhoeaceae (APG, 2009). *A. barbadensis* (syn. *Aloe vera*) is a short-stemmed succulent herb. It is light green with white spots and grows 1-4 feet tall. The leaves are 20-50 cm long, 3-5 cm wide at the base, tapering to a pointed tip. *Aloe vera* has a rhizome-root system (Rajeswari *et al*., 2012) and are shallow-rooted (Ryczkowski, 2020). Traditionally, the leaf juice is used to treat intestinal ulcers, catarrh and gynaecological problems (Idu *et al*., 2014). Similarly, Ross (1999) reported that fresh leaf juice is taken orally to treat stomach ulcers and heal wounds. The leaf, passed over a flame, can be used to manage skin irritations such as ringworm and eczema. The root of *A. barbadensis* is used to treat constipation and impotence

(Adodo, 2012). It is used as a purgative, appetite-stimulant, emmenagogue and for managing colds, piles, asthma, cough and jaundice in an ayurvedic formulation (Joseph and Raj, 2010). Among the Ifa Nkari people of Akwa Ibom State, Nigeria, the root of *A. barbadensis* is popularly used to treat low libido (Erhabor *et al*., 2013).

However, the absence of toxicological information on the root of *A. barbadensis* prompted this investigation. In this study, the toxicity or safety evaluation of *A. barbadensis* root was limited to the functional indices of the liver and kidney of male Wistar rats. The use of male Wistar rats was necessitated following the folkloric use of *A. barbadensis* root as an aphrodisiac in Nigeria, which has been scientifically validated elsewhere (Erhabor and Idu, 2017). Thus, this study was conducted to provide information on *A. barbadensis* root extract's toxicity in male rats.

Materials and methods

Collection, authentication, and extraction of plant material: The fresh roots of *Aloe barbadensis* were collected in Okene town, Kogi State, Nigeria. The plant was authenticated by Mr. G. Ibhanesebhor, Herbarium Unit, Obafemi Awolowo University, Ile-Ife, Nigeria, with voucher number IFE17004, where the plant was deposited. The roots were initially rinsed in running water and placed on laboratory tables to dry at room temperature. The roots were further dried and the plant material transferred to an oven set at 40 ºC for 10 minutes before grinding to powder. The fine powdered plant material (2000 g) was extracted with 5000 mL of ethanol using a soxhlet extractor. The extract was concentrated to dryness using a water bath (HH-S Water Bath; Searchtech Instruments) set at an average temperature of 50 ºC. The yield of the ethanolic extract was determined using the formula:

% yield= weight of extract/weight of powder sample x 100/1

Animal grouping and extract administration: A total of healthy sixty (60) male Wistar rats of 150-270 g body weight obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Nigeria, were utilized for this study. The animals were kept in the ventilated animal house of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Nigeria, for acclimatization with optimum conditions (temperature, 25 °C; photoperiod, 12 h of natural light and 12 h of dark). The animals had unrestricted access to water and were fed with standard commercial pellets. The Ethical Committee on Experimental Animal Use and Care of the Faculty of Life Sciences, University of Benin, Nigeria, reviewed and approved this study's protocol (LSC15101). The sixty (60) male rats were randomized into five groups (groups A, B, C and D) of 15 and given treatment orally. Group A was administered the diluent (2 mL of distilled water) while groups B, C and D were given 100, 200 and 400 mg/kg body weight of *A. barbadensis* root extract respectively, using an orogastric tube. Five (5) rats each from all the groups were sacrificed after 1¼ h of administering the respective doses of the extract on Days 1, 7 and 14. The animals were handled following the International Guiding Principles for Biomedical Tesearch involving animals as outlined by the Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science (CIOMS and ICLAS, 2012).

Preparation of serum: As outlined by Yakubu *et al*. (2005), the modified procedure was employed to prepare the serum. Blood was collected on days 1, 7 and 14, respectively. Briefly, under chloroform anesthesia, the rats' stomachs were cut open to expose the internal organs with the aid of sterile forceps and scissors. After that, blood was collected via cardiac puncture using a 5 mL syringe and needle per animal into appropriately labelled clean non-coagulant sample bottles. The sample bottles were left at room temperature for 10 minutes to clot. The bottles were centrifuged at 3000 rpm for 10 minutes using a laboratory centrifuge. The collected sera were aspirated with Pasteur pipettes into clean, dry sample plain bottles and used within 12 h of preparation for the biochemical assays.

Determination of biochemical parameters

Liver function tests: The protocols, as described by Reitman and Frankel (1957), were used in determining the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the collected serum. Alkaline phosphatase (ALP) level was assessed using the colorimetric endpoint method described in the manufacturer's assay kit (Teco Diagnostics, U.S.A). The method of Jendrassik and Grof (1938) was used to estimate the direct bilirubin concentration, while the technique of Doumas *et al*. (1971) was used to measure the albumin concentration. As outlined in the manufacturer's kit (Randox Laboratories Limited, UK), the Biuret method was used to ascertain the total protein.

Renal function test: The renal function parameters (creatinine, urea, potassium, calcium, chloride, sodium and phosphorus) were determined. The level of creatinine in the serum of the rat was measured using the colourimetric method described by Bartels *et al.* (1972), while the method described by Weatherburn (1967) and outlined in the manufacturer's kit (Randox Laboratories Limited, UK) was used to ascertain the urea concentration. The potassium and calcium concentrations were assessed utilizing the manufacturer's kits (Teco Diagnostics, USA and Randox Laboratories Limited, UK). The chloride concentration was ascertained following the modified method of Skeggs and Hochestrasser (1964). The modified method of Trinder (1951) and Maruna (1958) was used to determine the amount of sodium in the rats' serum. The phosphorus concentration was assayed using the protocol described by Garnst and Try (1980); Munoz *et al*. (1983).

Data analysis: Data were presented as mean ± SEM of the appropriate replicates. One Way ANOVA was done to compare means of different groups, and Duncan, multiple range tests, to ascertain the differences among various means and the interaction between the variables using the SPSS 15.0 computer software package. Differences at P<0.05 or P<0.01 were considered statistically significant.

Results

Extract yield: The 2000 g of the extracted root plant material yielded 6 % (124 g) of the ethanol extract.
Effect of the root extract of A. barbadensis on alkaline phosphatase (ALP): The tested doses of *A. barbadensis* extract and the control (distilled water) were noticed to be non-significantly different (P>0.05) from each other on Days 1, 7 and 14 of treatment. The Control, 200 and 400 mg/kg Dose Groups on Day 1 were significantly different from the same Dose Groups on Days 7 and 14, as depicted in Table 1. The oral administration of the extract showed an increase in ALP on Day 1, with a corresponding increase in doses. In contrast, on Days 7 and 14, there was a decrease in the ALP level as the dose increased.

Table 1: Effect of ethanol extract of *A. barbadensis* on alkaline phosphatase concentration (IU/L) of male rats.

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	$115.67^{\#\#}$±4.39	$27.55^{\#}$±1.04	$42.47^{\#}$±10.1	**P<0.01
100 mg/kg	75.19±19.51	88.81±23.62	42.98±12.06	NS
200 mg/kg	$92.79^{\#\#}$±16.87	$61.39^{\#}$±11.88	$33.11^{\#}$±3.26	*P<0.05
400 mg/kg	$108.46^{\#\#}$±18.9	$58.48^{\#}$±6.7	$28.67^{\#}$±5.67	**P<0.01
P-value	P>0.05	P>0.05	P>0.05	

DW-Distilled water; Values are expressed as means±SEM, n=5. ***P<0.01, *P<0.05-Significant, P>0.05 - Not Significant. Different superscript letters (in columns) show that the mean is significant from others. The same $^{\#}$(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows.*

Effect of the root extract of A. barbadensis on aspartate aminotransferase (AST): A non-significant increase in AST at the tested doses was observed on Day 1 as the reverse was noticed on Day 14, with a corresponding rise in doses (Table 2). All tested Dose Groups, including controls on Days 7 and 14, were not significantly different (P>0.05) from each other. The 100 mg/kg group was substantially different from the 200 and 400 mg/kg groups and controls.

Table 2: Effect of ethanol extract of *A. barbadensis* on aspartate aminotransferase concentration (U/l) of male rats

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	29.75^{a}±0.61	29.51±2.46	36.92±8.36	NS
100 mg/kg	13.63^{b}±2.5	30.43±4.98	27.76±11.97	NS
200 mg/kg	27.5^{a}±3.89	28.4±5.59	23.83±6.47	NS
400 mg/kg	33.05^{a}±1.39	32.41±3.31	24.66±2.75	NS
P-value	**P<0.01	P>0.05	P>0.05	

DW-Distilled water; values are expressed as mean ± SEM, n=5. ***P<0.01, *P<0.05-Significant, P>0.05 - Not Significant. Different superscript letter(s) (in columns) shows that the means are significantly different from others. NS - No Significant difference in days across the rows.*

Effect of the root extract of A. barbadensis on alanine aminotransferase (ALT): The effect of the extract on ALT levels in the male rats after days 1, 7 and 14 are displayed in Table 3. All tested dose groups, including controls on days 7 and 14, were found not significantly different (P>0.05) from each other. On day 1, the 200 mg/kg dose group was found significantly different (P<0.05) from other test groups, including the control.

Table 3: Effect of ethanol extract of *A. barbadensis* on alanine aminotransferase concentration (U/l) of male rats

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	12.6^{b}±1.68	8.83±1.07	8.03±1.65	NS
100 mg/kg	12.42^{b}±1.03	14.08±1.09	10.67±2.83	NS
200 mg/kg	16.53^{a}±3.76	13.34±1.83	9.01±3.02	NS
400 mg/kg	$7.1^{b\#}$±1.84	$13.79^{\#\#}$±1.68	$5.41^{\#}$±0.62	*P<0.05
P-value	*P<0.05	P>0.05	P>0.05	

DW-Distilled water; Values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Different superscript letters (in columns) show that the mean is significant from others. The same #(in rows) shows no significant difference across the sampled means across the days. NS – No Significant difference in days across the rows*

Effect of the root extract of A. barbadensis on total bilirubin: Table 4 shows the activity of the extract on the total bilirubin of the male rats on the different days of testing. The tested Dose Groups, including control on Days 1, 7 and 14, were found not significantly different (P>0.05) from each other. The highest total bilirubin concentration of 2.25 mg/dl was recorded on Day 1 at 200 mg/kg.

Table 4: Effect of ethanol extract of *A. barbadensis* on male rats' total bilirubin concentration (mg/dl)

Groups	Day 1	Day 7	Day 14	P-value
DW (DW)	0.66±0.09	0.4±0.04	0.7±0.11	NS
100 mg/kg	1.12±0.28	0.38±0.06	0.72±0.17	NS
200 mg/kg	2.25±1.39	0.34±0.07	0.53±0.08	NS
400 mg/kg	$0.70^{\#\#}$±0.07	$0.37^{\#}$±0.05	$0.43^{\#}$±0.04	**P<0.01
P-value	P>0.05	P>0.05	P>0.05	

DW-Distilled water; All values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. The same #(in rows) shows no significant difference across the sampled means across the days. NS – No Significant difference in days across the rows.*

Effect of the root extract of A. barbadensis on direct bilirubin: The non-significant effect (P>0.05) of the extract on direct bilirubin concentrations on all the days of treatments is depicted in Table 5. The 100 and 400 mg/kg dose groups were also non-significant in their effect on total bilirubin on different testing days.

Table 5: Effect of ethanol extract of *A. barbadensis* on direct bilirubin concentration (mg/dl) of male rats.

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	$0.24^{\#}$±0.08	$0.48^{\#}$±0.08	$1.02^{\#\#}$±0.22	**P<0.01
100 mg/kg	0.28±0.06	0.53±0.18	0.8±0.39	NS
200 mg/kg	$0.30^{\#}$±0.1	$0.29^{\#}$±0.05	$0.75^{\#\#}$±0.1	**P<0.01
400 mg/kg	0.3±0.05	0.52±0.08	0.52±0.1	NS
P-value	P>0.05	P>0.05	P>0.05	

DW-Distilled water; Values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Same #(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows*

Effect of the root extract of A. barbadensis on albumin: Table 6 revealed that the extract increased the male rat's albumin levels compared to the control, though statistically not significant. The administration of the 400 mg/kg of the extract recorded the highest concentration of 6.02 g/dl of albumin on day 7. The extract given to the tested groups, including control on days 1, 7 and 14, were found to have a non-significant effect on albumin levels.

Table 6: Effect of ethanol extract of *A. barbadensis* on male rats' albumin concentration (g/dl).

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	2.66±0.13	2.38±0.25	2.02±0.11	NS
100 mg/kg	$2.68^{\#\#}$±0.1	$1.89^{\#}$±0.19	$1.66^{\#}$±0.35	*P<0.05
200 mg/kg	$2.5^{\#\#}$±0.22	$2.01^{\#}$±0.09	$1.37^{\#}$±0.36	*P<0.05
400 mg/kg	2.99±0.09	6.02±4.08	1.77±0.46	NS
P-value	P>0.05	P>0.05	P>0.05	

DW-Distilled water; Values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Same #(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows*

Effect of the root extract of A. barbadensis on total protein: The total protein concentrations of the male rats orally given the ethanol extract of *A. barbadensis* are presented in Table 7. The effect of the extract on the same groups' total protein concentrations at different days was found to have a non-significant effect.

Table 7: Effect of ethanol extract of *A. barbadensis* on male rats' total protein concentrations (g/dl)

Groups	**Day 1**	**Day 7**	**Day 14**	**P-Value**
Control (DW)	7.32±0.4	5.69^{b}±0.37	6.78±0.53	NS
100 mg/kg	6.46±0.89	7.18^{a}±0.39	4.77±10	NS
200 mg/kg	5.81±1.12	7.3^{a}±0.44	4.46±0.85	NS
400 mg/kg	7.45±0.46	7.24^{a}±0.56	5.74±0.98	NS
P-value	P>0.05	*P<0.05	P>0.05	

DW-Distilled water; Values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Different superscript letters (in columns) show that the mean is significant from others. NS –No Significant difference in days across the rows*

Determination of creatinine concentrations: The creatinine values obtained after oral administration of the extract are shown in Table 8. The results revealed that the male rats' creatinine levels were not significantly different among the tested groups and control on days 1 and 14. The effect of the extract on creatinine concentrations of the same groups was found to be non-significant on different days.

Table 8: Effect of ethanol extract of *A. barbadensis* on male rats' creatinine concentrations (mg/dl)

Groups	**Day 1**	**Day 7**	**Day 14**	**P-Value**
Control (DW)	0.89±0.78	2.44^{a}±0.81	0.74±0.51	NS
100 mg/kg	0.94±0.51	0.41^{b}±0.13	0.62±0.2	NS
200 mg/kg	0.99±0.63	0.26^{b}±0.12	0.1±0.03	NS
400 mg/kg	0.84±0.2	2.67^{a}±0.26	1.56±0.81	NS
P-value	P>0.05	**P<0.01	P>0.05	

DW-Distilled water; Values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Different superscript letters (in columns) show that the mean is significant from others. NS –No Significant difference in days across the rows*

Determination of urea concentrations: Table 9 revealed that the extract increased urea concentration in a dose-independent manner. The effect of the extract on urea was not significant (P>0.05) among the tested doses and against the controls on days 1 and 7. On day 14, the 400 mg/kg and the control (distilled water) groups were significantly different (P<0.01) from the 100 and 200 mg/kg dose groups.

Table 9: Effect of ethanol extract of *A. barbadensis* on urea concentrations (mg/dl) of male rats

Groups	**Day 1**	**Day 7**	**Day 14**	**P-Value**
Control (DW)	96.61$^{\#\#}$±15.53	59.3$^{\#}$±5.11	61.57$^{a\#}$±3.82	*P<0.05
100 mg/kg	94.23$^{\#\#}$±11.5	61.57$^{\#}$±8.74	49.88$^{b\#}$±6.72	*P<0.05
200 mg/kg	78.08±17.20	48.67±5.53	41.30^{b}±3.7	NS
400 mg/kg	93.60±15.71	56.12±4.45	59.23^{a}±2.66	*P<0.05
P-value	P>0.05	P>0.05	**P<0.01	

DW-Distilled water; All values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Different superscript letters (in columns) show that the mean is significant from others. Same $^{\#}$(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows.*

Determination of potassium concentrations: As depicted in Table 10, potassium concentrations increased non-significantly (P>0.05) on days 1, 7 and 14 in all doses, including control. The extract at 400 mg/kg recorded the highest potassium concentration of 6.88 mEq/L.

Table 10: Effect of ethanol extract of *A. barbadensis* on potassium concentrations (mEq/L) of male rats

Groups	Day 1	Day 7	Day 14	P-values
Control (DW)	$4.00^{\#}$±0.43	$2.35^{\#}$±0.83	$6.65^{\#\#}$±0.67	**P<0.01
100 mg/kg	$5.01^{\#}$±0.33	$4.51^{\#}$±0.33	$6.86^{\#\#}$±0.75	*P<0.05
200 mg/kg	4.46±0.57	3.84±0.42	3.41±0.62	NS
400 mg/kg	3.3±0.47	3.08±0.94	6.88±2.69	NS
P-values	P>0.05	P>0.05	P>0.05	

DW-Distilled water; Values are expressed as Means±SEM, n=5. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Same $^{\#}$(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows*

Determination of calcium concentrations: Table 11 showed that the oral administration of the extract to the male rat had no significant effect on the rats' calcium concentrations in all treated groups on days 1, 7 and 14. The concentrations of calcium at 400 mg/kg on days 1, 7 and 14 were significantly different (P<0.01) from each other.

Table 11: Effect of ethanol extract of *A. barbadensis* on calcium concentrations (mg/dl) of male rats

Groups	Day 1	Day 7	Day 14	P-value
Control (DW)	$5.66^{\#}$±1.16	$14.46^{\#}$±1.61	$7.26^{\#\#}$±2.26	**P<0.01
Viagra (5mg/kg)	$7.09^{\#}$±0.83	$16.92^{\#\#}$±0.64	$6.21^{\#}$±1.5	**P<0.01
100 mg/kg	$7.81^{\#}$±1.04	$14.39^{\#\#}$±1.63	$12.2^{\#}$±1.61	*P<0.05
200 mg/kg	$5.81^{\#}$±0.8	$15.94^{\#\#}$±2.33	$12.76^{\#\#}$±1.87	**P<0.01
400 mg/kg	$6.39^{\#}$±0.67	$18.19^{\#\#\#}$±0.45	$12.86^{\#\#}$±2.87	**P<0.01
P-value	P>0.05	P>0.05	P>0.05	

DW-Distilled water; All values are expressed as Means±SEM of five animals in each group. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Same $^{\#}$(in rows) shows no significant difference across the sampled means across the days.*

Determination of chloride concentrations: The effect of the extract on chloride levels of the male rats, as displayed in Table 12, was not significant (P>0.05) among the tested doses and control on days 1 and 7. The 100 mg/kg of the extract increased the chloride levels significantly (P<0.05) compared to the other tested doses and control on day 14. The lowest chloride concentration (71.27±8.05 mEq/L) was at the 200 mg/kg dose, while the highest of 104.69±1.81 mEq/L was at the 400 mg/kg dose on day 1.

Table 12: Effect of ethanol extract of *A. barbadensis* on chloride concentrations (mEq/L) of male rats

Groups	Day 1	Day 7	Day 14	P-Value
Control(DW)	$108.46^{\#\#}$±3.15	$86.91^{\#\#}$±2.74	$80.73^{b\#\#}$±4.42	**P<0.01
100mg/kg	97.37±7.40	85.63±5.4	97.64^{a}±5.27	NS
200mg/kg	$97.71^{\#\#}$±6.47	$98.64^{\#\#}$±2.18	$71.27^{b\#}$±8.05	*P<0.05
400mg/kg	$104.69^{\#\#}$±1.81	$95.73^{\#}$±1.24	$81.27^{b\#\#}$±3.49	**P<0.01
P-value	P>0.05	P>0.05	*P<0.05	

DW-Distilled water; All values are expressed as Means±SEM of five animals in each group. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Different superscript letters (in columns) show that the means are significant from others. Same $^{\#}$(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows.*

Determination of sodium concentrations: Table 13 showed the extract's effect on the male rats' sodium levels. The effect was not significant (P>0.05) among the tested doses and the control on days 1, 7 and 14. Of all the tested doses, 100 mg/kg of the extract showed the highest concentration of 186.57±69.18 mEq/L of sodium.

Table 13: Effect of ethanol extract of *A. barbadensis* on sodium concentrations (mEq/L) of male rats

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	$228.54^{\#\#}$±14.29	$84.47^{\#}$±9.92	$106.94^{\#}$±23.52	**P<0.01
100 mg/kg	186.57±69.18	69.91±17.35	93.00±9.4	NS
200 mg/kg	156.91±40.52	67.95±14.38	94.29±16.43	NS
400 mg/kg	145.11±36.54	63.36±5.13	89.48±17.85	NS
P-value	P>0.05	P>0.05	P>0.05	

DW-Distilled water; All values are expressed as Means±SEM of five animals in each group. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Same #(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows*

Determination of phosphorus concentrations: The phosphorus concentrations increased dose-dependence on days 1 and 7, as displayed in Table 14. The 100 mg/kg of the extract increased phosphorus significantly on day 1compared to other dose groups and control.

Table 14: Effect of ethanol extract of *A. barbadensis* on phosphorus concentrations (mg/dL) of male rats

Groups	Day 1	Day 7	Day 14	P-Value
Control (DW)	5.87^{b}±1.12	3.59±0.69	6.52±1.25	NS
100 mg/kg	8.24^{a}±1.43	4.72±0.44	5.87±1.06	NS
200 mg/kg	3.67^{b}±0.94	4.45±1.12	4.86±0.73	NS
400 mg/kg	$3.3^{b\#}$±1.33	$4.17^{\#}$±0.34	$9.01^{\#\#}$±1.36	**P<0.01
P-value	*P<0.05	P>0.05	P>0.05	

DW-Distilled water; All values are expressed as Means±SEM of five animals in each group. ***P<0.01-Highly significant, *P<0.05-Significant, P>0.05-Not Significant. Different superscript letters (in columns) show that the mean is significant from others. Same #(in rows) shows no significant difference across the sampled means across the days. NS –No Significant difference in days across the rows*

Discussion and conclusion

The liver and kidney functional capacity of male Wistar rats was evaluated following the assessment of useful biochemical indices or "markers." Reports show that a significant change in any organ function's biochemical indices will impair the normal function of the organ (Afolayan and Yakubu, 2009). Again, many "marker" enzymes, including phosphatases, dehydrogenases and transferases found in the serum and do not originate from the extracellular fluid, imply tissue damage. This may be due to leakage; thus, some of these biomolecules find their way into the serum via disruption of cell membranes (Panda, 1989). Therefore, serum enzyme levels are valuable tools in clinical diagnosis and toxicological studies (Ashafa *et al*., 2009).

The administration of the ethanol extract of *A. barbadensis* at the various doses showed a non-significant effect on the marker enzymes and other biochemical indices of the liver. There were no significant changes (p>0.05) in ALP, AST and ALT (Table 1, 2 and 3), suggesting that there was no cellular damage to the plasma membrane of the male rats. This is similar to the liver functional indices (ALT and AST) results obtained by Amber *et al*. (2021) on the effect of *Aloe vera* gel (1 and 1.5%) introduced to the drinking water of broilers. The concentrations of other biochemical markers such as total bilirubin, direct bilirubin, albumin, and total proteins in the serum are valuable tools for determining any liver impairment (Yakubu *et al*., 2005). That these biomarkers (total bilirubin, direct bilirubin, albumin and total proteins) levels (Table 4, 5, 6 and 7) were not significantly (p>0.05) altered by the ethanolic extract of *A. barbadensis* indicates that the secretory functions of the liver were not impaired on the days of examination. However, the total protein level (Table 7) was found to be significantly (p<0.05) different at all treated doses from the control on day 7 as against days 1 and 14, where there was no change.

Renal function indices are used to assess the functional capacity of the various parts of the kidneys' nephrons (Abolaji et al., 2007). The concentrations of these markers (creatinine, urea and electrolytes) can therefore be used to monitor the adverse effect of a compound/ plant extract on the tubular and/ or the glomerular part of the kidney (Ashafa *et al*., 2009). It was observed that the extract had no significant (p>0.05) effect on renal function indices investigated (Tables 8-14). This suggests that the extract probably had an unremarkable effect on the nephrons' functioning at the tubular and glomerular levels. However, isolated cases of significant changes in some renal function indices were noticed. There

was a significant change at 100 and 200 mg/kg in the level of creatinine on day 7 (Table 8) and urea on day 14 (Table 9) while phosphorous (Table 14) and chloride (Table 12) on day1 and 14 at 100 mg/kg compared to the control. These changes do not suggest any form of renal impairment as they were significant reductions rather than an increase which are adjudged pathologically unremarkable; though, a consistent significant decrease in urea indicates an impairment in the functional capacity of the nephron (Yakubu *et al*., 2003) which indicates an abnormality in the physiological excretion of urea most likely due to a non-renal factor. It was observed that the extract exerted a significant ($p<0.01$) effect on urea at the lower doses (100 and 200 mg/kg) as against the highest dose and was noticed on day14 of the extract administration (Table 9). These changes were similar to a previous report by Amidu (2008), where the root extract of *Sphenocentrum jollyanum* on renal function indices was observed. The differences were not considered test drug-related since they were of small magnitude. They were also not dose-related (observed at the lower doses but not at the highest dose) as they occurred on specific days of the extract examination.

The toxicological study indicates that the extract was relatively safe for consumption at the administered doses. There was no significant deleterious effect on the functional indices of the liver and kidney of the male rats. Overly, no significant localized systemic toxicity was noticed. Still, there is a need for caution when using the extract of *A. barbadensis* root for oral remedies following its potential selective ability to alter specific biochemical parameters in male rats. Further studies on the effect of chronic administration of the root of *A. barbadensi*s are recommended.

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References

Abolaji AO, Adebayo AH, Odesanmi OS: Effect of ethanolic extract of *Parinari polyandra* (Rosaceae) on serum lipid profile and some electrolyte in pregnant rabbits. Res J Med Plant 1: 121-127. 2007.

Adebayo JO, Adesokan AA, Olatunji LA, Buoro DO, Soladoye, AO: Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. Biokemistri 17: 45. 2005.

Adodo A: Nature power: A Christian Approach to Herbal medicine. Third edition, PAX Herbal Clinic & Research Laboratories, Ewu, Edo State, Nigeria. 2012.

Afolayan AJ, Yakubu MT: Effect of *Bulbine natalensis* Baker stem extract on the functional indices and histology of liver and kidney of male Wistar rats. J Med Food 12(4): 814 – 820. 2009.

Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ: Toxicological evaluation of the aqueous extract of *Felicia muricata* Thunb. leaves in Wistar rats. Afr J Biotechnol 8(6): 949 – 954. 2009.

Amber K, Nofel R, Ghanem R, Sayed S, Farag SA, Shukry, M, Dawood MA: Enhancing the growth rate, biochemical blood indices, and antioxidative capacity of broilers by including *Aloe vera* gel in drinking water. Front Vet Sci 1193. https://doi.org/10.3389/fvets.2020.632666

Amidu N: An evaluation of the central and sexual behavioural effect and toxicity of the root extract of *Sphenocentrum jollyanum* Pierre (Menispermaceae). Ph.D. thesis. Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. 211p. 2008.

APG (The Angiosperm Phylogeny Group): An update of an angiosperm phylogeny group classification for the orders and families of flowering plants. APG 111. Bot J Linn Soc 161: 105-121. 2009.

Bartels H, Böhmer M, Heierli C: Serum creatinine determination without protein precipitation. Clin Chim Acta 37:193-197. 1972.

Betram GK: Introduction to Toxicology: Occupational and Environmental in Basic and Clinical Pharmacology, Seventh Edition. Appleton and Lange, 946p. 1998.

CIOMS, ICLAS: International guiding principles for biomedical research involving animals. Council for International Organization of Medical Sciences (CIOMS) and International Council for Laboratory Animal Science (ICLAS). [Online-Available at: http://www.cioms.ch/images/stories/CIOMS/IGP2012.pdf]. 4p. 2012.

Coles EH: "Veterinary Clinical Pathology". W.B. sanders, Philadelphia, USA, pp.10 - 42. 1986.

De Gruchy GC: Chemical Haematology in Medical Practice. Blackwell Scientific Publication, Oxford, London. pp. 33-57 1976.

Doumas BT, Watson WA, Biggs HG: Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31: 87-96 .1971.

Enas EA: Cholesterol made easy: The good, bad and the ugly. CADI Research, USA. pp. I-3.1999.

Erhabor JO, Idu M, Udo FO: Ethnomedicinal urvey of medicinal plants used in the treatment of male infertility among the Ifa Nkari people of Ini Local Government Area of Akwa Ibom State, Nigeria. Res J Recent Sci 2(ISC-2012): 5-11. 2013.

Erhabor JO, Idu M: Aphrodisiac potentials of the ethanol extract of *Aloe barbadensis* Mill. root in male Wistar rats. BMC Complement Altern Med 17(1): 1-10. 2017.

Garnst O, Try K: Determination of serum-phosphate without deproteinization by ultraviolet spectrophotometry of the phosphomolddic acid complex. Scand. J Clin Lab Investig 40: 483- 486.1980.

Idu M, Ataman JE, Akigbe AO, Omogbai EKI, Amaechina F, Odia, EA: Effects of *Stachytarpheta jamaicensis* (L) Vahl on wistar rats:serum biochemistry and ultrasonography. J Med Sci 6(4): 646-649. 2006.

Idu M, Erhabor JO, Ovuakporie-Uvo O: Ethnomedicinal plants used by the Idoma people – Benue State, Nigeria. Am J Ethnomed 1(1): 72-88. 2014.

Jendrassik L, Grof P: Colorimetric Method of Determination of bilirubin. Biochem. Z 297: 81-82. 1938.

Joseph B, Raj SJ: "Pharmacognostic and phytochemical properties of *Aloe vera* Linn- an overview". Int J Pharm Sci Rev Res 4(2): 106-110. 2010.

Maruna RFL: Determination of serum sodium by the magnesium uranyl acetate. Clinica Chimica Acta 2: 581-585.1958.

Munoz MA, Balon, M, Fernandez C: Direct determination of inorganic phosphorus in serum with a single reagent. Clin Chem 29: 372-374. 1983.

Panda NC: Kidney. In: Textbook of Biochemistry and Human Biology (2nd edition). Talwer, G. P., Stivastava, L. M. and Moudgil, K. D. (eds). Prentice-Hall of Indian Private Limited pp.276 – 292. 1989.

Pousset JL: Medicinal plants used as a source of raw material for the pharmaceutical industry, problems and prospects. In: Drug Production from National Products, Proceedings of the Seventh International Symposium. DRPU. OAU, Ife, Nigeria.pp.37-41. 1988.

Rajeswari R, Umadevil M, Sharmila Rahale C, Pushpa R, Selvavenkadesh S, Kumar KS, Debjit B: *Aloe vera:* The miracle plant, its medicinal and traditional uses in India. J Pharmacogn Phytochem 1(4): 118-124. 2012.

Range HP, Dale M, Ritter SM: Reproductive aging in the male Brown-Norway rat: a model for the human. Endocrinology 133: 2773-2781. 1995.

Reitman S, Frankel S: A colorimetric method for determination of serum glutamateoxaloacetate and pyruvate transaminases. Am J Clin Pathol 28: 56-61. 1957.

Ross IA: Medicinal plants of the World: Chemical constituents, traditional and modern medicinal uses. Humana press, Totawa New Jersey. 415p. 1999.

Ryczkowski A: Plant Parts of *Aloe vera*. Available at: https://homeguides.sfgate.com/plant-parts-aloe-vera-68299.html, 2020.

Skeggs LT, Hochstrasser HC: Thiocyanate (colorinmetric) method of chloride estimation. Clin Chem 10: 918-920. 1964.

Sofowora EA: Medicinal plants and Traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 289p. 1993.

Tietz NM: Clinical Guide to Laboratory Tests, Second edn. W.B. Saunders Company, Philadelphia, USA. 931p. 1990.

Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW: Beneficial effects of cholesterol lowering therapy on the coronary heart disease. N Engl J Med 332: 481.1995.

Trinder P: A rapid method for the determination of sodium in serum. Analyst 76: 596-599.1951

Trinder P: Determination of glucose in blood using glucose oxidase with on alternative oxygen receptor. Ann Clin Biochem 6: 24-27.1969.

Weatherburn MW: Colourimetric Urease-Berthelot method of urea estimation. Anal Chem 39: 971-974. 1967.

Williams N, Levine RF: The original, development and regulation of megakaryocytes. Br J Haematol 52: 173. 1982.

Yakubu MT, Bolbis LS, Lawal M, Akanji MA: Evaluation of selected parameters of rat and kidney function following repeated administration of yohimbine. Biokemistri 15(2): 50 – 56. 2003.

Yakubu MT, Akanji MA, Oladiji AT: Aphrodisiac potentials of the aqueous extract of *Fadogia agresitis* (Schweinf.Ex Hiern) stem in male albino rats. Asian J Androl 7(4): 399-404. 2005.

Yakubu MT, Akanji MA, Oladiji AT: Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia argrestis* stem. Pharmacogn Mag 3: 34. 2007.

Yakubu MT, Akanji MA, Oladiji AT: Alteration in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia argrestis* stem. Res J Med Plant 2: 66. 2008

Yakubu MT, Afolayan AJ: Effect of aqueous extract of *Bulbine natalensis* Baker stem on haematological and serum lipid profile of male wistar rats. Indian J Exp Biol 47: 283 – 288. 2009.