THE IMPACT OF *VISCUM ALBUM* LEAF EXTRACT ON SOME BIOCHEMICAL PARAMETERS, LIVER AND KIDNEY FUNCTION INDICES IN FEMALE RATS

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ABTRACT

Viscum album Linn, (mistletoe) a Loranthacea is used in the management of different diseases. This study assessed the impact of *V. album* leaf aqueous extract on some biochemical and functional indices in female rats. Twenty female Wistar rats with average weight of 164.69 ± 2.02 g were assigned to study groups B, C and D and, a control (Group A) with five animals per group. Group A received 0.5ml of distilled water, while animals in groups B, C and D were administered with 50, 100, and 200 mg/kg body weight of the extract respectively, by oral, daily and for 30 days. The extract significantly ($p<0.05$) decreased follicle stimulating hormone, caused elevation of the luteinizing hormone and progesterone in serum of the animals. Serum levels of triglyceride, total cholesterol and low-density lipoprotein were also reduced, whereas high density lipoprotein, Superoxide dismutase activity and the vitamin C concentration were increased ($p<0.05$) significantly. Activity of liver ALT, AST and GGT activities in serum, serum total protein, albumin and globulin, conjugated bilirubin as well as K^+ and HCO_3^- levels were not significantly ($p>0.05$) different in extract-treated and the control. *Viscum album* extract however, aggravated some indices of liver (13%) and the kidney (17%) functions. In conclusion, the overall results with 70% positive impact obtained, showed that *V. album* may be used as adjunct to therapy in management of anovulatory infertility and metabolic syndromes but need further toxicological investigations

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INTRODUCTION

Viscum album Linn (mistletoe) is a general name used to describe numerous species of hemi-parasitic plants which belong to the family Loranthaceae [1]. It is identified by various names locally, such include "*Afomo onisannan"* (South-Western), "*Kauchi*" (North-Western) and "*Apari*" (South-Eastern) Nigeria respectively [1, 2, 3]. In German, V. *album* is referred to as "*Mistel", "Vogelmistel*", "*Leimmistel*" and "*gui commun*". "*Gui de druids"* in French and bird lime in Europe [4]. They are mostly found growing on various deciduous trees such as *Azadirachta indica* (neem), *Theobroma cacao* (cocoa), *Cola nitida* (kola nut), *Pentaclethra macrophylla* (oil bean) and *Albizia lebbeck*, the lebbeck trees [2, 5, 6].

The *V. album* is regarded as medicinal plant [7]. It is used in different forms such as decoction, fluid extracts, infusion and tea bags to manage several diseases globally, either in folk and orthodox medicine or as a complementary therapy [4, 7]. In the ethnomedicinal uses of mistletoe for instance, the European mistletoe was reported to be used to manage diabetes and high blood pressure. Mistletoe extracts were explored as an unorthodox oncology therapy in Germany [4, 7]. In Japan, mistletoe was used to treat hypertension, spasms of the cardiac muscle, rheumatic pain and threatened abortion and tea prepared from mistletoe leaves was used in India to treat diabetes [4, 7]. In Nigeria, decoction of mistletoes stem is used as management option for diabetes mellitus, hypertension, stroke as well as menstrual disorder and infertility [2, 8]. Furthermore, antioxidants, antidiabetic, antihypertensive, anti-inflammatory as well as hepato-protective in hepatocellular damage and reno-protective against nephrotoxicity in rats are some of the pharmacological activities of the plant which have been reported [7, 9, 10].

Numerous and diverse phytochemicals found mostly in four biochemical classes which include the alkaloids, glycosides, polyphenols and terpenes have also been identified in the different species of *V. album* [5, 6, 7]. These bioactive constituents have been shown to be responsible for varying biochemical and biological effects of the plant. These pharmacologically active compounds possess direct or indirect therapeutic effects and are included as medicinal agents in common drugs like aspirin, digoxin, quinine, and opium, thus contributing to the therapeutic evidence of the plant [4]. Many studies have investigated the phytotherapeutic potentials of *Viscum album* in different experimentally-induced animal models disease conditions and organ dysfunction. For instance, 400 mg/kg methanol extract was shown to demonstrate antidiabetic effect, it decreases

hyperglyceamia by reducing fasting blood sugar in rats [11]. In another study [12], 100 mg/kg aqueous extract restored irregular oestrus cycle and polycystic ovary morphology in polycystic ovarian syndrome rats. A 350 mg/kg ethanol extract was also reported to prevent oxidative stress, liver injury and the hepatic dysfunction in chlorpyrifos treated rats [13].

However, there is scanty of scientific information on the effect of *V. album* extracts on biochemical and functional indices in normal rats, which may provide insights to some biochemical activities of the plant. Hence, this study aimed to assess the impact of *V. album* leaf extract on concentration of selected biochemical parameters, liver and kidney function indices in healthy female Wister rats.

MATERIALS AND METHODS

Study area

Osun grove is located along the bank of Osun River in Osogbo capital city of Osun State, South Western Nigeria. It is located on a latitude of 7°45'05.9"N and longitude of 4°33'03.9"E, 250km north of Lagos. The grove has a land size of 75 hectares and it is about 350 meters above sea level. It houses hundred shrines, sculptures and serves as world heritage site [14, 15].

Ethical clearance

The experimental protocols were approved by Ethical Committee on the Use, Care and Handling of laboratory animals, University of Ilorin Ethical Review Committee (UERC), (Reference: UIL/UERC/12/68DM002, Number: UERC/ASN/2015/222).

Sample collection

Leaves of *V. album* grown on kola nut host tree were collected from Osun grove, Osogbo, south-western Nigeria, after due identification and authentication with a voucher specimen number (UILH/ 001/1210) in the Herbarium Unit, Department of Plant Biology, University of Ilorin, Nigeria.

Experimental animal

Twenty female Wister rats with average weight of 164.69 ± 3.02 g used were obtained from the Department of Biochemistry, University of Ilorin, Nigeria. The animals were housed in plastic cages, placed in well ventilated Animal House, under normal atmospheric conditions and a 12h dark/light cycle. The animals were freely fed with standard rat pellets (Premier Feed Mills Co., Ltd., Nigeria) and tap water throughout the duration of the experiment

Extract preparation

The leaves were washed, air dried at room temperature (30ºC) for two weeks and pulverized using an electric blender (Master Chef Model MC – BL1970, USA). A 500 g portion of the pulverized leaves were macerated in 1 L of distilled water for 48 h with the solution being shaken at intervals. The resulting solution was then filtered (whatman no 1 filter paper) and the filtrate was freeze dried at 65ºC (J9897/2 Lyotrap lyophilizer, LTF Scientific Greenfield, Oldham, UK). A 13.58 g of the crude extract was produced, corresponding to 9.05 % yield. This was reconstituted in distilled water to give the doses of 50, 100 and 200 mg/kg body weight which were administered [12].

Groups of animals and extract administration

After acclimatization for two weeks, the animals were assigned to groups B, C and D and a control (Group A) with five animals per group. The animals were administered orally with the extract daily for 30 days as follows:

Group A = 0.5 ml of distilled water (Control)

Group B = 50 mg/kg body weight of *V. album*

Group C = 100 mg/kg body weight of *V. album*

Group D = 200 mg/kg body weight of *V. album*

A 41.20, 82.30 and 164.70 mg of the extract were separately dissolved in 5ml of distilled water and 1ml of the solution was administered orally to each rat in groups B, C and D respectively to obtain the required 50, 100 and 200 mg/kg body weight dosage.

Preparation of serum and liver supernatant

At the end of the experimental period, animals in their estrus phase were anesthetized with diethyl ether before being sacrificed, 5 ml of venous blood was collected from each animal into plain bottles and allowed to clot at 30ºC for 45 min., after full clot retraction, the sample was centrifuged at 850 g for 10 min. The supernatant serum was pipetted into another plain bottle using a Pasture pipette, sealed tightly, and frozen at -20ºC till when it was used for biochemical tests. The animals were swiftly dissected, the liver was excised and blotted with blotting paper. A section (1 g) was homogenized in ice-cold 0.25 M sucrose solution (1:4 w/v). The homogenate of the liver was centrifuged at 1000 g

for 10 min, and the supernatant was used to assay the liver marker enzymes.

Determination of serum gonadotropins and progesterone

Serum, follicle stimulating hormone (FSH), luteinizing hormone (LH) and the progesterone were estimated using a commercial assay kits (Monobind Inc. Lake Forest, USA). The procedure outlined in the manufacturer's manual packed with the assay kits adopted the principle of enzyme immunoassay (ELIZA) and was followed.

Serum lipid profile analysis

Triglyceride (TG), total cholesterol (tcholesterol) and the high-density lipoprotein cholesterol (HDL-C) fraction were analyzed with commercial reagent kits (Agappe Diagnostics, Switzerland GmbH). The procedure outlined in the manufacturer's manual enclosed in the assay kits was used, it adopted the colorimetric based CHOD-PAP method [13]. The low-density lipoprotein cholesterol (LDL-C) and the atherogenic index were then computed [14] using the formula:

$$\text{LDL (mg/dl)} = \text{tCholesterol} - \text{HDL} - \frac{\text{TG}}{5}$$

$$\text{Atheriogenic Index} = \frac{\text{LDL-C}}{\text{HDL-C}}$$

Determination of serum superoxide dismutase activity and vitamin C concentration

Activity of superoxide dismutase (SOD) and the vitamin C were estimated by standard spectrophotometric method [18, 19].

Determination of selected liver maker enzymes

The activities of alanine and aspartate aminotransferase (ALT and AST) were determined as described by Reitman and Frankel [20], and the method of Szasz [21] was adopted for gamma glutamyl transferase (GGT) assay.

Determination of some serum proteins and bilirubin

Serum total protein, albumin and bilirubin were respectively determined by spectrophotometric methods [22, 23, 24]. The globulin fraction was calculated by subtracting the serum albumin concentration from the total serum protein concentration: Globulin (g/dl) = Total Protein – Albumin and the conjugated bilirubin also from the total bilirubin gives the unconjugated bilirubin [25].

Determination of some serum electrolytes, urea and creatinine

The method described by Tietz, [25] was used for the determination of sodium, potassium, chloride and the bicarbonate ions as well as urea concentration in the serum using the procedure outlined in the commercial reagent kit manual. Sodium and potassium were determined using the flame photometer, spectrophotometry for chloride ions and urea and the titration method for bicarbonate ion concentration. Creatinine was determined by spectrophotometry using method of Veniamin and Varkirtzi-Lemonias [26].

Data analysis

Data are presented as mean of five determination ± standard error of mean (SEM). Significance of difference among groups was determined by One-way analysis of variance (ANOVA), Post Hock multiple comparison analysis by the Duncan's Test and $p < 0.05$ was accepted as significant. The chart was drawn using graph pad prism 5.

RESULTS

Oral administration of the *Viscum album* leaves extract for 30 days significantly ($p<0.05$) decreased follicle stimulating hormone (FSH) in the serum of the animals, with a high LH: FSH ratio of 2.88 (1.70 ± 0.06: 0.59 ± 0.05) and 1.42 (1.76 ± 0.05: 1.24 ± 0.17) in the 50 and 100 mg/kg compared to 0.60 (1.84 ± 0.12: 3.07 ± 0.06) for the distilled water control group as shown in Table 1. The extract also significantly decreased ($p<0.05$) the serum levels of triglyceride, total cholesterol and the low-density lipoprotein, whereas the high-density lipoprotein was significantly increased ($p<0.05$) with a profound effect in the 100 mg/kg group compared to the control (Table 2). Activity of superoxide dismutase and vitamin C concentration in serum of the animals that received 100 and 200 mg/kg extract were also statistically ($p<0.05$) elevated (Figure 1 & 2). Furthermore, activity of liver ALT, the AST and GGT activities in serum and liver (Table 3), serum total protein, albumin and globulin, conjugated bilirubin levels (Table 4) as well as the serum K^+ and HCO_3^- (Table 5), were all not significantly different ($p>0.05$) in the groups administered with the extract and the control group.

However, compared with the control, 50 mg/kg of the extract significantly ($p<0.05$) increased activity of ALT in serum of the animals by 11% (Table 3), while the unconjugated bilirubin was decreased by 52% (Table 4). It also elevated Na^+ (9%) and the urea level by 136% (Table 5). The 100 mg/kg of the extract significantly increased serum ALT by 7% (Table 3), urea by 130 % and then reduced the creatinine by 12% as shown in Table 5. Furthermore, animals that received 200 mg/kg of the extract showed increase of 23% in total bilirubin (Table 4) and a decrease in creatinine by 8% (Table 5).

Table 1: Serum gonadotropic and progesterone hormones concentrations of female rats after administration of *V. album* leaves extract

		***V. album* (mg/kg) body weight**		
Parameters	**Control**	**50**	**100**	**200**
FSH (mIU/ml))	3.07 ± 0.06	0.59 ± 0.05	1.24 ± 0.17	2.17 ± 0.44
LH (mIU/ml))	1.84 ± 0.12	1.70 ± 0.06	1.76 ± 0.05	0.76 ± 0.16
Progesterone (ng/ml)	8.13 ± 0.14	8.00 ± 0.06	6.60 ± 0.16	6.89 ± 0.33

Values are mean ± SEM, (n=5). Values across the rows with different superscripts are significantly different ($p < 0.05$). The FSH (Follicle stimulating hormone) and LH (Luteinizing hormone).

Table 2: Serum lipid profile of female rats after administration of *V. album* leaves extract

		***V. album* (mg/kg) body weight**		
Parameters (mg/dl)	**Control**	**50**	**100**	**200**
Triglyceride	160.63 ± 5.38	146.41±6.19	115.49 ± 3.27	125.40 ± 3.91
Total cholesterol	88.16 ± 4.25	59.51 ± 4.90	48.36 ± 2.05	81.53 ± 4.51
HDL	11.14± 0.88	14.57 ± 1.75	18.94 ± 3.10	17.62 ± 3.08
LDL	44.89± 3.620	15.74 ± 4.53	6.32 ± 1.940	39.18 ± 5.50
Atherogenic index	4.03± 0.34	1.07 ± 0.29	0.34 ± 0.12	2.37 ± 0.5 0

Values are mean ± SEM, (n=5). Values across the rows with different superscripts are significantly different ($p < 0.05$). HDL (High density lipoprotein), LDL (Low density lipoprotein).

Table 3: Activities of alanine, aspartate and gamma glutamyl transferase in the liver and serum of female Wister rats after administration of *V. album* leaves extract

			***V. album* mg/kg body weight**		
Enzymes (U/L)	**Tissue**	**Control**	**50**	**100**	**200**
ALT	Liver	76.04 ± 4.87	68.82 ± 4.4	71.62 ± 6.57	73.35 ± 4.77
	Serum	3.97 ± 0.20	4.42 ± 0.39	4.23 ± 0.44	3.89± 0.22
AST	Liver	41.52 ± 4.70	41.94 ± 3.78	39.75 ± 4.40	39.56 ± 4.63
	Serum	5.31 ± 0.48	5.03 ± 0. 74	5.39 ± 0.29	5.22 ± 0.35
GGT	Liver	21.10 ± 1.60	25.98 ± 2.75	24.75 ± 2.24	24.84 ± 2.41
	Serum	3.47 ± 0.52	2.55 ± 0.28	3.01 ± 0. 70	3.40 ± 0.37

Values are mean ± SEM, (n=5). Values across the rows with different superscripts are significantly different ($p < 0.05$)

Table 4: Selected plasma protein and bilirubin of female Wistar rats after administration of *V. album* leaves extract

		***V. album* (mg/kg) body weight**		
Parameters	**Control**	**50**	**100**	**200**
Total protein	6.97 ± 0.56 ■	7.48 ± 0.38 ■	6.78 ± 0.60 ■	7.18 ± 0.47 ■
Albumin	3.25 ± 0.73 #	4.25 ± 0.20 #	3.79 ± 0.37 #	4.00 ± 0.41 #
Globulin	3.05 ± 0.45 ▼	3.24 ± 0.33 ▼	2.99 ± 0.58 ▼	3.18 ± 0.46 ▼
Total bilirubin	1.41 ± 0.08 ⁂	1.26 ± 0.10 ⁂	1.29 ± 0.15 ⁂	1.73 ± 0.25 ⁂
Conjugated bilirubin	1.18 ± 0.13 ♦	1.14 ± 0.10 ♦	1.12 ± 0.19 ♦	1.36 ± 0.15 ♦
Unconjugated bilirubin	0.23 ± 0.07 ♠	0.11 ± 0.03 ▲	0.16 ± 0.06 ♠	0.37 ± 0.12 ♠

Values are mean ± SEM, (n=5). Values across the rows with different superscripts are significantly different ($p < 0.05$). Plasma protein concentrations (g/dl) and the bilirubin (mg/dl)

Table 5: Selected electrolytes, urea and creatinine concentrations in serum of female Wistar rats after administration of *V. album* leaves extract

Parameters		***V. album* (mg/kg) body weight**		
(Mmol/L)	**Control**	**50**	**100**	**200**
Na^+	124.80 ± 1.92 ▼	132.60 ±3.70 ▌	127.40 ±3.43 ▼	130.00 ± 4.36 ▼
K^+	3.70 ± 0.34 ⁂	3.94 ± 0.27 ⁂	4.62 ± 0.14 ⁂	4.38 ± 0.32 ⁂
HCO^-_3	26.60 ± 1.34 #	24.60 ± 3.58 #	27.40 ± 1.52 #	28.20 ± 0.84 #
Cl^-	93.20 ± 4.76 ▲	103.60 ± 3.21 ■	100.41 ± 3.91 ■	100.80± 2.39 ■
Urea	2.31 ± 0.16 ⁑	5.46 ± 0.22 *	5.32 ± 0.52 *	2.90 ± 0.47 ⁑
Creatinine	116.90± 5.77 ♦	113.38 ± 3.77 ♦	103.10 ± 2.50 ■	107.10 ±1.50 ▲

Values are mean ± SEM, (n=5). Values across the rows with different superscripts are significantly different ($p < 0.05$)

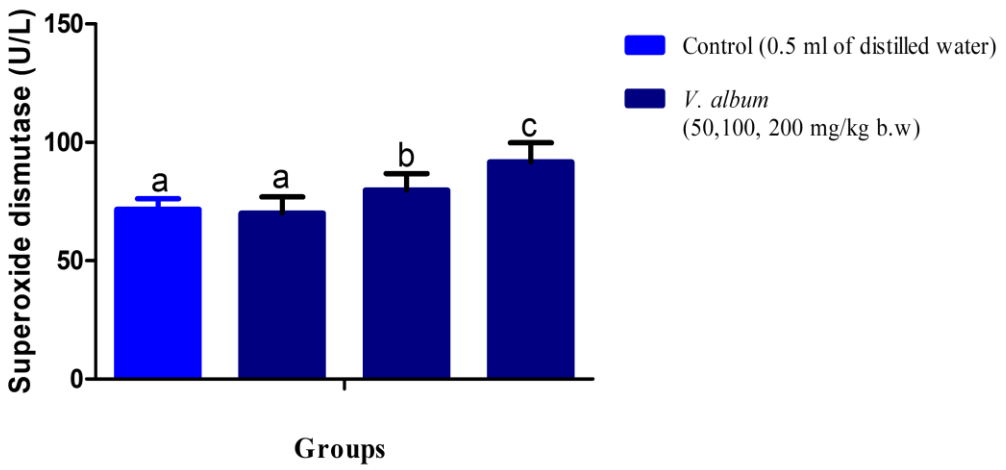

Figure 1: Activity of super oxide dismutase in serum of female Wistar rats after administration of *V. album* extract. Bars are mean ± SEM, (n=5). Bars with different letters are significantly different ($p<0.05$), b.w.: body weight

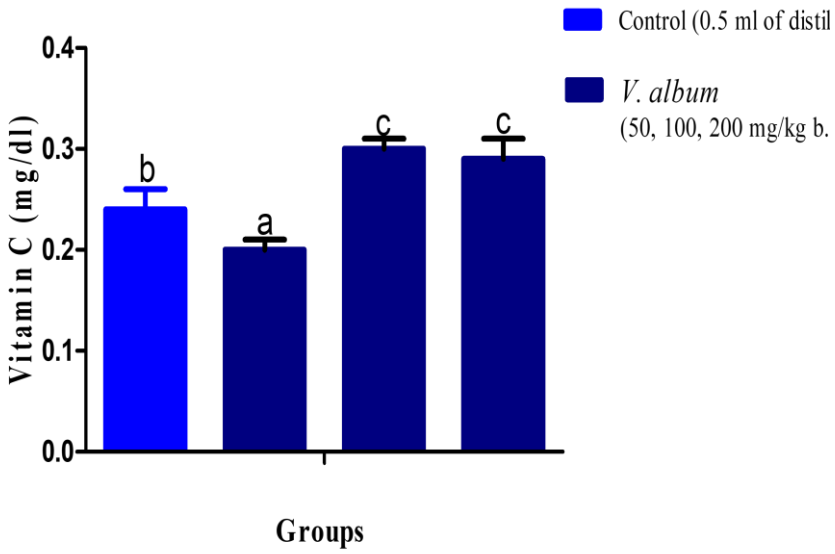

Figure 2: Vitamin C concentration in serum of female Wister rats after administration of *V. album* extract. Bars are mean ± SEM, (n=5). Bars with different letters are significantly different ($p<0.05$), b.w.: body weight

DICUSSION

Follicle stimulating hormone (FSH) and the luteinizing hormone (LH) are gonadotropic hormones which by feedback mechanisms are released, effect and regulate optimum production and action of oestrogen and progesterone as would be required during the sexual cycle [27]. Mid cycle decreases in FSH with corresponding increase in LH leading to the LH surge is essential for ovulation to occur [27]. The 50 mg/kg of the extract which produced high level of luteinizing hormone and a slight elevation of the progesterone in estrus phase (mid cycle / ovulation stage) of the oestrous cycle, the sexual cycle in rats [28] is an indication that the extract, may subsequently promote ovulation. The possible biochemical stimulating effect of *V. album* on LH, which is associated with ovulation, may therefore, partly account for therapeutic potential of the plant in infertility due to anovulation. This corroborate fertility potential of the plant earlier reported [2, 7].

Enhanced exogenously (dietary) or endogenously accumulation of abnormal lipid in the serum, leading to elevated serum triglyceride, total cholesterol and their related lipoprotein fractions (LDL-C and HDLC-C) ratio are indication of high atherogenic index which is a risk factor for cardiovascular diseases [29]. Reduced TG, Cholesterol, LDL and the increased HDL shown by the extract therefore, implies that *V. album* could decrease the extent of lipogenesis and then the associated incidence of atherosclerosis, high blood pressure and other cardiovascular diseases. An elevated, high density lipoprotein cholesterol to low density lipoprotein-cholesterol ratio (HDL-C: LDL-C) correlates positively with lower incidence of coronary artery diseases [29]. Hence, the increase in the high-density lipoprotein-cholesterol concentration following administration of the various doses of the extract in this study indicated that the plant may protect against cardiovascular diseases. Reducing the extent of fat accumulation and the sodium potassium ion ratio, which may enhance relaxation of blood vessel walls may justify the phytotherpeutic evidences of the plant as antiobesity, antidiabetic or antihypertensive [7, 27, 29].

Alanine and Aspartate aminotransferase (ALT and AST) as well as gamma glutamyl transferase (GGT) are enzymes found in the liver [30]. Chemicals and drugs which may also include the *V. album* can cause alteration in their activities [31, 32], changes are used in diagnosis of liver damage, dysfunction or diseases, treatment exposure or outcome and in toxicological evaluation [30], for instance, increase in ALT and AST are used as surrogate marker for liver injury, elevated activity of AST and GGT in serum aid indication of hepatocellular damage, which are used in the differential diagnosis of obstructive jaundice and myocardial infarction [13, 30,]. Therefore, insignificant increase or decrease in the activity of ALT, AST and the GGT in liver of the animals' given extract implies that the extract could not have caused adverse liver injury, hepatocellular damage and alterations in cell membrane permeability which may lead to liver dysfunction and thereby causing abnormal *de novo* metabolism of these enzymes.

Total protein consists of the soluble proteins in blood, albumin and globulins are the major components. Total protein, albumin and bilirubin are metabolized in the liver and are used as indices of the liver's synthetic and excretory functions. Albumin is synthesized in the liver and can be broken down to its amino acid components, which are then used to produce other proteins. Serum albumin concentration is used as an index of albumin turnover [33, 34]. Therefore, the absence of change in the serum levels of total protein, albumin, and globulin of the animals after administration of extract suggests that the liver's ability to synthesize these plasma proteins may not have been compromised. The elevated unconjugated bilirubin may however be an indication of bile duct obstruction and decreased bile release to the small intestine for elimination. This could have led to bile buildup in the liver and consequently the accumulation of unconjugated bilirubin as seen in the blood of the study animals [35], similar elevating effect of the highest dose could not be detected on either of conjugated or unconjugated bilirubin as corroborated by insignificant effect of this dose on the bilirubin fractions, this imply that insult on the liver and biliary tracts affecting the metabolism of bilirubin could not be appropriately established. Hence, further toxicity studies are recommended.

The kidney performs homeostatic and excretory functions involving regulation of electrolyte balance, removal of urea, and the creatinine, through glomerular filtration of plasma water, selective tubular reabsorption and the secretion of electrolytes, fluids and solutes. Thus, electrolytes, urea and the creatinine are utilized to assess glomerular filtration rate, tubular reabsorption and secretory functions of the nephron [36, 37, 38]. Increase reabsorption of sodium in the proximal convoluted tubules as indicated by

elevation in serum is usually accompanied by Cl^-, HCO_3^- and water reabsorption, this can cause water retention, increase intravascular fluid volume and high blood pressure in renal arteries [39], which can progressively modulate renal injury and loss of function. Potassium supplementation leading to elevated plasma level has vasodilation effect, promoting muscle contraction, blood flow and thereby reducing the pressure against blood vessel walls [40]. Therefore, elevated K^+ and the decrease Na^+ levels in serum of animals that were administered with the 100 and 200 mg/kg body weight suggest that these doses might cause natriuresis, accompanied by salt and water excretion and then, Na^+ reduction in the body of the animals. This may lead to decrease blood pressure in the renal arteries and may also support antihypertensive impact of the plant.

Impaired renal reabsorption can cause electrolyte derangement and accumulation of toxic metabolites such as urea in the blood. This is corroborated by high level of urea observed in the 50 mg/kg. The increased urea may also be due to competitive excretion of *Viscum album* and the urea in the nephron. Certain constituents in the extract may compete with urea for the tubular transport mechanism, thereby decreasing secretion of the urea in the renal tubules. This may have resulted in the delayed excretion and consequently, the elevation in plasma levels. The highest dose used however restored the renal malfunction affecting urea metabolism as evidenced from the lack of change in urea level from the control. Serum creatinine is one of the indices utilized to assess glomerular filtration rate and the impact of a medication on the nephron [36, 37, 41]. Therefore, the reduction of creatinine due to the extract administration may be indicative of an increased glomerular filtration rate and renal excretion mediated by some metabolites of the extract.

Super oxide dismutase (SOD) and vitamin C are *in vivo* antioxidant indices, the SOD is an enzymatic antioxidant which could prevent superoxide anion (O^*) and oxygen radical (O_2^*) [42], increase in the serum activity of this enzyme caused by the extract may implies possible activation of SOD which can facilitate reduction of superoxide anions to a non-radical oxygen molecule. Vitamin C is a water-soluble essential micronutrient which act as a non-enzymatic antioxidant by scavenging of peroxyl radicals [42]. Boost the immune system and combat susceptibility to infectious diseases, low plasma and tissue vitamin C concentration correlate well with many diseases [43]. Therefore, increase in these biochemical compounds by the extract may contribute to antioxidant enhancing potential of the plant, increased antioxidant status can protect cell membranes from free radicals damaging effect and oxidative stress implicated in different pathological conditions. Positive impacts of *V. album* extract on concentration of the biochemical indices which were observed in this study may justify the therapeutic effects of the plant. The dose specific abnormal alterations which were shown in serum ALT activity, Na^+, Cl^-, urea and creatinine concentrations may be an indication for possible toxicity potential associated with the *V. album* extract.

CONCLUSION

The mid cycle high LH and FSH ratio involvement in ovulation and possibly in anovulatory infertility, decreased serum lipids and elevation of serum potassium which may promote antihypertensive therapy as well as the increased antioxidant status protection demonstrated by *Viscum album* leaf extract may justify the therapeutic evidences of the plant in the management of numerous metabolic or chronic diseases. Further studies are recommended to evaluate the toxicological effects of the plant.

REFERENCES

1. SALEH, I., MAIGANDI, S.A., HADU, M.I., ABUBAKAR, M.I & SHEU, A.U. (2015). Uses and chemical component of mistletoe (*V. album*) obtained from different species of trees. *Dutse Journal of Agricultural and Food Security,* **2**(1): 008-012.
2. ADESINA, S.K., ILLOH., C.H., IMOH, I.J. & IMO, I.J. (2013). Africa mistletoes (Loranthaceae) ethnopharmacology, chemistry, and medicinal values: an update. *Africa Journal of Traditional Complement and Alternative Medicine,* **10**(3): 161-170.
3. TIZHE, T., ALONGE., S.O. & RAMATU E.A. (2016). Mistletoe presence on five tree species of Samaru area, Nigeria. *African Journal of Plant Science,* **10**(1): 16-22.
4. BRAHMA, N.S., CHAITRALI, S., DANIJEL, G., DALIP, K.U. & SRINI, V.K. (2016). *Viscum album:* a potent phytotherapeutic agent with multifarious phytochemicals, pharmacological properties and clinical

evidence. *RSC Advances, Royal Society of Chemistry*, **6**(28): 23837-23892.

5. IBE, F.C., IBE, B.O. & ENYOH, C.E. (2019). Trace metal, FTIR and phytochemical analysis of *Viscum album* leaves harvested from *Pentaclethra macrophylla*. *World News of Natural Sciences*, **25**: 61-71.
6. OSENI, F.A., IBRAHIM, S.A., AHMAD, H.D., ABUBAKAR, M.S. & ABBAS, S.R. (2022). Phytochemicals in methanolic leaf extracts of *Tapinanthus globiferus* from *Azadirachta indica* and *Albizia lebbeck*: a comparative analysis of some pharmacological potentials. *Bayero Journal of Pure and Applied Sciences*, **13**(1): 223-229.
7. SZURPNICKA, A., KOWALCZUK, A. & SZTERK, A. (2020). Biological activity of mistletoe in vitro and in vivo studies and mechanism of action. *Archives of Pharmacology Research*, **43**:593-629.
8. OGUNMEFUN, T.O., FASOLA, T.R., SABA A.B. & ORIDUPA, O.A. (2013). The ethnobotanical, phytochemical and mineral analyses of *Phragmanthera incana* (Klotzsch) a species of mistletoe growing on three plant hosts in south-western Nigeria. *International Journal of Biomedical Science Research*, **9**(1): 33-40.
9. ESIN, S.C., HASSAN, T.., OZGUR, I.C, NIGER, Y. & IBRAHIM, H.C. (2017). Methotrexate induced nephrotoxicity in rats; protective effect of mistletoe (*Viscum album* L.) extract. *Complementary Medicine Research*, **24**: 364-370. Doi: 10.1159/000468984
10. ROSELINE, E., BIOSA, N. & TAMUNO-EMINE, D.G. (2018). Therapeutic effect of *Viscum album* against CCL4-induced hepatotoxicity in male albino rats. *World Journal of pharmaceutical and Medical Research*, **4**(7): 14-20.
11. OSADEBE, P.O. & JOHNSON – AJINWO, O.R. (2021). An Investigation into the seasonal variation of the phytochemical and anti-diabetic properties of the eastern Nigerian specie of African Mistletoe (*Loranthus micranthus*) sourced from *Kola acuminata*. *Diabetes Complications*, **5**(2): 1-5.
12. OSENI, F.A., SALAU, A.K., AFOLABI-BALOGUN, N.B. & BALOGUN, E.A. (2019). Effects of aqueous extract of *Viscum album* leaf on some biochemical parameters and ovarian morphology of oestradiol valerate-induced polycystic ovarian syndrome rats. *International Journal of Biological and Chemical Sciences*, **13**(6): 2521-2532.
13. MILOSEVIC, M.D., MASKOVIC, P.Z., STANKOVIC, V.D., PAUNOVIC, M.G., MITIC, M.N., MITIC, M.M OGNJANOVIC, B.I. (2022). Protective effects of *Viscum album* L. leaf extract on chlorpyrifos-induced hepatotoxicity in Wister rats. *Journal of King Saud University-Science*, **34**:101957.
14. NATIONAL COMMISSION FOR MUSEUMS AND MONUMENTS (NCMM). (2005). Nomination to the World Heritage List of Osun-Osogbo Sacred Grove, Osogbo, Osun State, Nigeria. Retrieved from https://whc.unesco.org/uploads/nominations/1118.pdf
15. OSEGALE, G., OMISORE, E. & GBADEGESIN, J.T. (2014). Exploratory survey on the maintenance of Osun Osogbo sacred grove, Nigeria. *African Journal of Hospitality, Tourism and Leisure*, **3**(2): 1–22.
16. FREDRICKSON, D.S., LEVY, R.I. & LEES, R.S. (1967). Fat transport in lipoproteins-an integrated approach to mechanisms and disorders. *New England Journal of Medicine*, **276:** 148 – 156.
17. FRIEDEWALD, W.T., LEVY, R.I. & FRIEDRICKSON, D. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clinical Chemistry*, **18:** 499- 502.
18. MISRA, H.P. & FRIDOVICH, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, **247**(10):3170-3175.
19. HENRY, R.J., CANNON, D.C. & WINKELMAN, J.W. (1974). Clinical Chemistry Principles and Techniques 2nd Ed. Harper and Row, Hugerstown, pp 1395–1398.
20. REITMAN, S. & FRANKEL, S. (1957). A colorimetric method for determination of

serum Glutamate, oxaloacetate and pyruvate transaminase. *America Journal of Pathology*, **28**(1): 56-63.

21. SZASZ, G. (1969). Kinetic photometric method for serum gamma-glutamyl transpeptidase. *Clinical Chemistry*, **15**(2): 124-136.
22. GORNALL, A.C., BARDAWILL, C.J. & DAVID, M.M. (1949). Determination of serum protein by means of biuret reaction. *Journal of Biological Chemistry*, **177**: 751-756.
23. DUMAS, B.T., WATSON, W.A. & BIGGS, H.G. (1971): Albumin Standards and Measurement of serum albumin with bromocresol green. *Clinical Chemical Acta*, **258**(1): 21-30.
24. EVELYN, K.A. & MALLOY, H.T. (1938): Micro determination of oxyhaemoglobin, methaemoglobin and sulphaemoglobin in a single sample of blood. *Journal of Biological Chemistry*, **126**: 655-661.
25. TIETZ, N.W. (1995). Clinical guide to laboratory tests. 4th Ed. Philadelphia: W. B. Saunders Company.
26. VENIAMIN, M.P. & VARKIRTZI-LEMONIAS, C. (1970). Chemical basis of the carbamidodi-Acetyl micro-method for estimation of urea, citrulline and carbamyl derivatives, *Clinical Chemistry*, **16**(10): 3-6.
27. ABASIAN, Z., ROSTAMZADEH A., MOHAMMADI, M., HOSSEINI, M. & RAFIEIAN-KOPAEI M. (2018). Review on role of medicinal plants in polycystic ovarian syndrome: pathophysiology, neuroendocrine signaling, therapeutic status and future prospects. *Middle East Fertility Society Journal*, **23**: 255-262.
28. PACCOLA, C.C., PESENDE, C.G., STUMPP, T., MIGRAGLIA, S.M. & CIPRIANO, I. (2013). The rat estrous cycle revisited: a quantitative and qualitative analysis. *Animal Reproduction*, **10**(4): 677 – 683.
29. MICHAEL, O.A., BIMBOLA, F.M & ROTIMI, O. (2019). The relationship between measures of obesity and atherogenic lipids among Nigerians with hypertension. *Malawi Medical Journal*, **3**: 193-197.
30. MALAKOUTI, M., KATARIA, A., ALI, S.K. SCHENKER, S. (2017). Elevated liver enzymes in asymptomatic patients-what should I do? *Journal of Clinical and Translational Hepatology*, **5**(4): 394-403.
31. YAKUBU, M.T. & NURUDEEN, Q.O. (2014). Toxicological effects of aqueous extract of *Cnestis ferruginea* (Vahl ex DC) root in paroxetine-induced sexually impaired male rats. *Journal of Investigational Biochemistry*, **3**(2): 39-45.
32. MATIC, K. & MIHALJEVIC PELE, A (2018). Drug induced toxic hepatitis associated with the combination of quetiapine and fluphenazine: A case report. *European Journal of Psychiatry*, **32**(4): 192-194.
33. LEVITT, D.G. & LEVITT, M.D. (2016). Human serum albumin homeostatis: a new look at the synthesis, catabolism, renal and gastrointestinal excretion and the clinical value of serum albumin measurements. *International Journal of General Medicine*, **9:** 229-255.
34. WADA, Y., SETO, N., KOMATSU, Y., TSUDA, M. & TAKEDA, Y. (2019). Plasma albumin redox state is responsive to the amino acid balance of dietary proteins in rats fed a low protein diet. *Frontiers in Nutrition*, **6:** 12 Doi. Org/103389/fnut.2019.00012.
35. MEMON, N., WEINBERGER, B.I., HEGYI, T. & ALEKSUNES, L.M. (2015). Inherited disorders of bilirubin clearance. *Pediatric Research*, **79**(3): 378-384.
36. ARAUJO, M. & DOI, S.Q. (2017). Editorial: Biomarkers in chronic kidney diseases (CKD). *Frontiers in Medicine*, **4**: 168. Doi: 10.3389/fmed.2017.00168.
37. KOVALCIKOVA, A., JANSAKOVA, K., GYURASZOVA, M., PODRACKA, L., SEBEKOVA, K. & CELEC, P (2018). Salivary creatinine and urea are higher in an experimental model of acute not chronic renal diseases. *Plos One*, **13**(7): e0200391.
38. WEINER, D., MITCH, E.W. & SANDS, M.J. (2015). Renal urea and ammonia nitrogen metabolism. *Clinical Journal of the American Society of Nephrology*, **10**: 1444-1458.
39. MANE, A.S. (2018). Correlation between serum sodium and potassium levels and risk of developing hypertension. *International Journal of Contemporary Medical Research*, **5**(11): k5-k9.

40. STARUSCHENKO, A. (2018). Beneficial effects of high potassium: contribution of renal basolateral K^+ Channels. *Hypertension*, (71): 1015-1022.
41. OSTERMANNI, M., KASHANI, K. & FORNI, G.L. (2016). The two sides of creatinine: both as bad as each other. *Journal of Thoracic Disease*, **7**: E628-E630.
42. IGHODARO, O.M. & AKINLOYE, O.A (2018). First line defense antioxidants-superoxide dismutase, catalase and glutathione peroxidase: their fundamental role in the entire antioxidant defense grid. *Alexandria Journal of Medicine*, **54**(4): 287-293.
43. CARR, A.C. & MAGGINI, S. (2017). Vitamin C and immune function. *Nutrients*, **9:** 1211.