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# Biochemical and histoarchitectural changes in the liver, kidney and heart after oral administration of *Viscum album* leaf extract in oestradiol valerate-treated female rats

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**ABSTRACT**

Effect of Viscum album leaf aqueous extract (VALEA) on biochemical parameters of oestradiol valerate (OV)-treated female rats was assessed. Group A rats received 0.5 ml of distilled water (control), the OV-treated rats (Groups B – F) were respectively administered 0.5 ml of distilled water, metformin (2.4 mg/kg bdwt), 50, 100 and 200 mg/kg bdwt of VALEA for 30 days. The OV treatment significantly decreased ($p<0.05$) liver and serum ALP, liver GGT and serum hexokinase, as well as levels of total bilirubin, urea, K+ and HDL. Also, activities of kidney ALP, liver and serum ALT, serum AST, serum GGT as well as unconjugated bilirubin, blood glucose, Na+, HCO3-, FFAs, triacylglycerols and VLDLs were elevated by OV treatment, which also induced liver fat and myocarditis. Significantly unaffected ($p>0.05$) by the OV treatment were liver AST, serum total proteins, albumin, globulin, conjugated bilirubin, creatinine, uric acid, Cl-, TC, atherogenic index and the kidney histoarchitecture. Administration of VALEA reversed the OV-induced biochemical alterations and the liver and cardiac histoarchitectural integrity in a manner that compared well with metformin in some parameters and doses. The results showed that VALEA has the potential to ameliorate OV-induced biochemical and histoarchitectural alterations in female rats.

**Keywords:** Liver, kidney, oestradiol valerate, Viscum album leaves, Loranthaceae, polycystic ovarian syndrome, metformin.

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## 1. Introduction

Hormonal imbalance, characterized by increased luteinizing hormone, hyperandrogenaemia, especially testosterone, as well as insulin resistance and hyperinsulinaemia are prominent features of polycystic ovarian syndrome (PCOS). This is accompanied by reproductive inadequacy and metabolic errors [1]. Diabetic mellitus and hypertension are common metabolic syndromes associated with PCOS condition due to insulin resistance-mediated impaired glucose metabolism and dyslipidaemia. Risk factors for pathogenesis of hypertension include electrolyte imbalance, especially the ratio of sodium to potassium, obesity, high atherogenic index and other lipid ratios [2-4]. Insulin regulates blood glucose homeostasis by promoting tissue glucose uptake, glycolytic breakdown or glycogenesis via glycogen synthase stimulation. The insulin receptor substrate (IRS) complex stimulates glucose absorption from the circulation by activating kinases and glucose transport protein 4 (GLUT-4) upon binding to the receptor. Impaired insulin receptor signalling pathway can cause insulin resistance and hyperinsulinaemia. This can result in hyperglycaemia and obesity, which are the pathophysiological core of PCOS [1,5].

Management options for PCOS usually involve combination therapy for reproductive inadequacy and the metabolic abnormality, such as combination of clomiphene citrate (an ovulation inducer) and metformin, an insulin sensitizer. These are scarce, expensive and with reported adverse effects [6,7], therefore, necessitating the screening of medicinal plants with reproductive and metabolic activities.

Viscum album L., (Loranthaceae) commonly called mistletoe in English, is referred to as Afomo, Kauchi and Osisi by the Yoruba, Hausa, and Igbo languages of Nigeria, respectively [8]. It is an evergreen shrub, 15–80 cm long, with oblong leaves that grows on the trunk of various deciduous host trees including citrus, cocoa and kola-nut. V. album is a semi-parasitic plant, in that it can photosynthesize but derive water and mineral elements from the host tree [9,10]. It is widely distributed in tropical and sub-tropical regions of Europe, United States, Australia, Asia and Africa, including Nigeria. The V. album plant is known as “cure all” and has been used for the management of different ailments like haemorrhoids, hypertension, diabetes mellitus, epilepsy and menstrual disorders such as oligomenorrhea and amenorrhea, both in traditional and complementary medicines [11]. The tea has also been used to manage stroke and it is believed to improve heart circulatory rates and blood circulation by the traditional healers [12]. Fertility potential, antidiabetic, blood pressure lowering and antihypertensive effects of the plant are well documented [10,11,13-16]. Mistletoe extract has also been shown to decrease serum lipids and ameliorate adverse effects on the morphology of the heart in triton Wr-1339 treated rats [17].

Aqueous extract of V. album leaves has been reported to contain essential inorganic elements such as potassium, calcium, zinc, manganese and selenium [18]. Other active compounds reported to be present in the plant include polysaccharides, amino acids and proteins, fatty acids, vitamin C, flavonoids, phenolic acids, alkaloids, terpenoids, phenylpropanoids, lectins and viscotoxins [10,12]. Antidiabetic, anti-epileptic, anticancer, immunomodulatory, antihypertensive as well as the antimicrobial activities of V. album leaf extract are well documented [10]. Viscothionin, an isolate from V. album has also been reported to stimulate the release of insulin from the pancreatic β-cell [16]. The immunomodulatory effect was attributed to the attenuation of tumour-induced immunosuppression of dendritic cells [19]. Furthermore, V. album leaf extract has been reported to exhibit hepato-protective and reno-protective activities in chemically-induced hepatocellular damage and nephrotoxicity in rats [20,21].

Oestradiol valerate (OV) is a fatty acid derivative of oestradiol 17β (oestrogen) and it is used as contraceptive. It is a pro-drug which, after administration, can be metabolized to the active oestrogen agonist such as ethinyloestradiol and valeric acid, the short chain fatty acid component. Enhanced conversion of the oestradiol metabolite to oestradiol can elevate oestradiol levels and increase hepatic steroid levels which, by feedback mechanisms, may cause desired oestrogenic effects in target tissues and/or undesirable effects on other organs and biochemical parameters [22,23]. These can include renal injury, hyperandrogenaemia and hyperlipidaemia.

However, the dearth of reports in the scientific literature on the effects of aqueous extract of V. album leaves on oestradiol valerate-treated rats necessitated this investigation. Therefore, this study assessed the effects of Visum album leaf aqueous extract on some biochemical and histopathological changes in the liver, kidney and heart of OV-treated rats.

## 2. Materials and Methods

### 1. Plant Material

Leaves of *V. album* were collected from kola-nut host tree at the Osun Grove, Osogbo, south-western Nigeria. Authentication of the leaves was carried out at the Herbarium Unit, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (UILH/ 001/1210) was deposited.

### 2. Animals

Thirty adult female rats (177.72 ± 2.08 g) were obtained from the Department of Biochemistry, University of

Ilorin, Ilorin, Nigeria. They were housed in standard plastic cages, in well-ventilated Animal House, maintained under the standard conditions of temperature (22.0 ± 3.0$^0$C), relative humidity (45 -50%) and a 12 h dark-light cycle. The animals had unrestricted access to rat pellets (Premier Feed Mills Co., Ltd., Nigeria) and tap water throughout the duration of the experiment.

### 3. Chemicals and Reagents

Oestraldiol valerate (OV) was a product of Merit Healthcare Pvt. Ltd., Mumbai**,** India. Assay kits used for total protein, albumin, bilirubin, urea, creatinine, uric acid, alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were products of Agappe Diagnostics, Cham-Switzerland GmbH. Triacylglycerol, total cholesterol (TC) and high-density lipoprotein (HDL) commercial assay kits used were products of Agappe Diagnostics, Switzerland GmbH. All other reagents used were products of Sigma Aldrich Ltd., Busch, Canada.

### 4. Preparation of Extract

The leaves of *V. album* were washed, air dried at room temperature (30°C) for two weeks and pulverized using an electric blender (Master Chef Model MC – BL1970, USA). A 200 g portion of the powder was extracted in 1 L of distilled water for 48 h. The resulting filtrate, which was lyophilized (J9897/2 Lyotrap lyophilizer) LTF Scientific Oldham Greenfield, UK), produced 23.49 g (11.75%) of the *V. album* leaf aqueous extract (VALEA). A 44.43, 88.86 and 177.72 mg of the extract were separately reconstituted in distilled water to give the respective 50, 100 and 200 mg/kg doses which were administered [18]. Equivalence of the ethnobotanical dose used for infertility was determined to arrive at the 200 mg/kg, a middle dose and a low dose, which were calculated half and one-quarter of the folkloric dose of 200 mg/kg body weight. The test doses were also below the LD50 values reported for Nigeria species of *V. album* [24]

### 5. Animal Grouping and Administration of Drug and Extract

After acclimatization for two weeks, animals were randomly assigned into six groups (A – F) with five rats per group. Animals in in the control group (Group A) did not receive injection or some other manipulations [25], they were administered with 0.5 ml distilled water orally, once daily throughout the 30 days experimental period. Single intramuscular injection of 2 mg/kg bdwt of OV was administered to animals (to induce PCOS) in groups B – F along with oral administration with 0.5 mL of distilled water, 2.4 mg/kg bdwt of metformin, 50, 100 and 200 mg/kg bdwt of VALEA, respectively, for 30 days. Only animals with confirmed PCOS were selected into groups B – F, which was confirmed as described previously [18]. 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).

### 6. Preparation of Serum and Tissue Supernatants

Twenty-four hours after the experimental period, animals were anaesthetized with halothane before being sacrificed. As previously described [26], 5 ml of venous blood was collected from each animal into plain bottles and allowed to clot at 30 ºC for 45 min before being centrifuged at 850 x *g* for 10 min. The serum was pipetted into another plain bottle using a Pasteur pipette and frozen at -20 ºC before being used for biochemical investigations. The animals were swiftly dissected, and the liver, kidney and heart were excised and blotted with tissue paper. A section (1 g) was homogenized in ice-cold 0.25 M sucrose solution (1:4 w/v). The homogenates of the liver and kidney were centrifuged at 1000 x *g* for 10 min, and the supernatants were used for the biochemical assays.

### 7. Biochemical Assays

Serum total protein (TP), albumin and bilirubin (BIL) were determined by adopting the methods described by Gornall *et al.* [27], Doumas *et al*. [28] and Evelyn and Malloy [29], respectively. Serum globulin concentration was calculated by subtracting serum albumin concentration from the TP concentration: Globulin (mg/dl) = TP – Albumin [30]. Urea, creatinine and uric acid (UA) were respectively determined according to standard procedures [30-32]. Activity of ALP was assayed by the method of Wright *et al.* [33], while ALT and AST were determined by the methods described by Reitman and Frankel [34]. The activity of GGT was assayed by adopting the procedure described by Szasz [35], hexokinase activity was determined as described by Brandstrup *et al.* [36] and glucose level by colorimetric glucose oxidase reaction (GODS) method of Trinder [37]. Free fatty acids (FFA), triglycerides, TC and the HDL were estimated using colorimetry-based CHOD-PAP method [38]. The LDL and atherogenic index (AI) were computed using the formula below [39]:

LDL (mg/dl) = (TC – HDL –TG)/5

AI = LDL/HDL

Sodium, potassium, chloride and the bicarbonate ions concentrations were also assayed according to standard procedures [30].

### 8. Histological Examination

A representative was selected from each group, 1 g section each from the liver, kidney and heart were collected and prepared for the microscopic examination as described by Junqueira *et al* [40]. Briefly, each tissue section collected were cut into smaller pieces (6mm) and fixed in 10 % formol saline for 24 h, then dehydrated with 100% ethanol in ascending grades (50%, 70%, 90% and 100%). The fixed portions were embedded in paraffin and cut into 5 µm thickness using a microtome. The thin sections were stained with haematoxylin (H), counter stained with eosin (E) and were then examined under a light microscope. The photomicrographs were captured at x100 magnification.

### 9. Data Analysis

The data were subjected to one-way analysis of variance with Duncan's Multiple Range Test to establish the differences between the means using IBM SPSS software (version 21.0). Values were considered significant at $p<0.05$. All the data were then expressed as mean ± SEM of five replicates.

## 3. Results and Discussion

Following OV treatment, liver and serum ALP were reduced, while activity in kidney was elevated significantly ($p<0.05$). Metformin administration significantly ($p<0.05$) reversed the changes in liver and kidney ALP activities, but did not affect the serum ALP activity ($p>0.05$) when compared with the group treated with OV+distilled water. Although, 200 mg/kg bdwt of VALEA attenuated the OV-induced decrease in serum ALP activity, other extract doses further reduced the liver and serum ALP below the control values (Table 1). VALEA also reversed the OV-induced elevation in the kidney ALP in a dose-independent manner (Table 1).

**Table 1** Activities of selected cellular enzymes in OV-treated rats administered VALEA

| Enzyme activity (IU/mg protein) | Control (distilled water only) | Oestradiol valerate (2 mg/kg body weight) | | | | |
|---|---|---|---|---|---|---|
| | | Distilled water | Metformin(2.4 mg/kg body weight) | *V. album* extract (mg/kg body weight) | | |
| | | | | 50 | 100 | 200 |
| Liver alkaline phosphatase | 33.87±0.34$^{b}$ | 32.04±0.74$^{c}$ | 35.30±0.64$^{a}$ | 24.84±0.70$^{e}$ | 27.43±0.71$^{d}$ | 32.17±0.70$^{c}$ |
| Kidney alkaline phosphatase | 28.02±0.49$^{d}$ | 66.72±1.40$^{a}$ | 23.33±0.30$^{e}$ | 26.14±1.72$^{d}$ | 31.41±0.19$^{c}$ | 48.40±4.35$^{b}$ |
| Serum alkaline phosphatase | 3.74±0.12$^{a}$ | 3.32±0.14$^{c}$ | 3.25±0.09$^{c}$ | 1.35±0.09$^{e}$ | 2.98±0.18$^{d}$ | 3.51±0.17$^{b}$ |
| Liver alanine aminotransferase | 43.94±0.15$^{b}$ | 47.51±0.36$^{a}$ | 37.24±0.14$^{c}$ | 44.44±0.19$^{b}$ | 43.75±0.20$^{b}$ | 35.04±0.12$^{d}$ |
| Serum alanine aminotransferase | 3.25±0.03$^{b}$ | 4.22±0.10$^{a}$ | 3.37±0.09$^{b}$ | 1.69±0.08$^{d}$ | 2.63±0.09$^{c}$ | 2.38±0.09$^{c}$ |
| Liver aspartate aminotransferase | 41.35±1.00$^{a}$ | 40.65±1.00$^{a}$ | 35.86±0.75$^{b}$ | 36.06±0.48$^{b}$ | 42.19±0.86$^{a}$ | 35.33±0.04$^{b}$ |
| Serum aspartate aminotransferase | 1.67±0.05$^{a}$ | 2.13±0.17$^{b}$ | 2.19±0.08$^{b}$ | 1.49±0.10$^{a}$ | 1.75±0.10$^{a}$ | 1.57±0.13$^{a}$ |
| Liver gamma glutamyl transferase | 22.36±0.61$^{b}$ | 13.52±1.89$^{c}$ | 12.19±0.90$^{d}$ | 34.20±0.61$^{a}$ | 23.92±0.68$^{b}$ | 22.62±1.69$^{b}$ |
| Serum gamma glutamyl transferase | 2.59±0.17$^{c}$ | 3.50±0.17$^{a}$ | 3.19±0.13$^{b}$ | 2.40±0.24$^{c}$ | 2.20±0.22$^{d}$ | 1.96±0.15$^{d}$ |
| *Serum hexokinase | 3.68±0.17$^{a}$ | 2.54±0.16$^{c}$ | 1.76±0.02$^{e}$ | 1.96±0.02$^{d}$ | 2.99±0.02$^{b}$ | 3.47±0.08$^{a}$ |

Values are mean ± SEM of five determinations. Means across the same row with different superscript letters are significantly different ($p<0.05$). *Hexokinase (µmol glucose phosphorylated/min/g protein).

Metformin and extract were also observed to have significantly reversed the OV-induced liver and serum ALT activities. The reduction was extract dose-dependent in the case of liver ALT (Table 1). Liver AST activity was not significantly ($p>0.05$) affected by OV and 100 mg/kg bdwt of VALEA; metformin and the other extract doses caused significant reduction in liver AST activities in the rats. In contrast, serum AST was elevated by OV and metformin when compared to the distilled water control, while all VALEA doses did not significantly affect serum AST when compared to the normal rats (Table 1).

Liver GGT was significantly reduced by OV treatment, which was further reduced by metformin. This trend was reversed by VALEA in a dose-dependent manner, though 50 mg/kg bdwt was still significantly higher than the normal control rats

(Table 1). OV-induced increase in serum GGT activity was attenuated by metformin and VALEA in a dose-dependent manner. The extracts performed better than metformin in this reversal (Table 1). Serum hexokinase activity was reduced by OV treatment, which was further reduced by metformin and 50 mg/kg bdwt of VALEA. However, administration of 100 and 200 mg/kg bdwt of VALEA reversed this trend, with the 200 mg/kg bdwt of VALEA being similar to the normal control rats (Table 1).

Serum TP, albumin, globulin and conjugated bilirubin (CBIL) were not significantly ($p > 0.05$) affected by any of the treatments in the course of this study (Table 2). Serum total bilirubin (TBIL) was reduced by OV, metformin and extracts, while OV-induced elevation of unconjugated bilirubin (UBIL) and blood glucose were reversed ($p<0.05$) by metformin and extracts (Table 2). As shown in Table 3, UA was unaffected significantly by any treatment; OV-induced urea reduction was restored by only 200 mg/kg bdwt of VALEA ($p<0.05$). Whereas, OV+distilled water and OV+metformin did not cause any significant ($p>0.05$) alterations in creatinine concentration, all the OV+extract doses caused significant ($p<0.05$) creatinine elevations (Table 3). Furthermore, OV-induced serum sodium elevation was significantly attenuated by metformin and all extract doses ($p < 0.05$); OV+distilled water, OV+metformin and OV+extract at 50 mg/kg bdwt significantly lowered serum potassium below the normal control value, while 100 and 200 mg/kg bdwt of VALEA significantly increased serum potassium above the normal control value (Table 3). While OV+100 and 200 mg/kg bdwt of VALEA significantly reduced serum chloride ions, only the 200 mg/kg bdwt of VALEA significantly reversed the OV-induced increase in serum bicarbonate ions (Table 3).

**Table 2** Selected liver function indices and blood glucose concentration of OV-treated female rats after oral administration of VALEA

| **Parameters** | **Control (distilled water only)** | **Distilled water** | **Oestradiol valerate (2 mg/kg body weight)** | | | |
|---|---|---|---|---|---|---|
| | | | **Metformin (2.4 mg/kg body weight)** | ***V. album* extract (mg/kg body weight)** | | |
| | | | | **50** | **100** | **200** |
| Serum total protein (g/dL) | 7.63±0.35$^{a}$ | 7.43±0.27$^{a}$ | 6.91±0.28$^{a}$ | 7.30±0.32$^{a}$ | 7.08±0.32$^{a}$ | 7.03±0.13$^{a}$ |
| Serum albumin (g/dL) | 4.29±0.37$^{a}$ | 3.93±0.07$^{a}$ | 3.81±0.28$^{a}$ | 3.74±0.20$^{a}$ | 3.58±0.15$^{a}$ | 3.60±0.30$^{a}$ |
| Serum globulin (g/dL) | 3.34±0.09$^{a}$ | 3.50±0.23$^{a}$ | 3.10±0.22$^{a}$ | 3.56±0.28$^{a}$ | 3.50±0.27$^{a}$ | 3.43±0.23$^{a}$ |
| Serum total bilirubin (mg/dL) | 1.45±0.06$^{a}$ | 1.32±0.02$^{b}$ | 1.30±0.07$^{b}$ | 1.04±0.12$^{e}$ | 1.26±0.03$^{c}$ | 1.21±0.05$^{d}$ |
| Serum conjugated bilirubin (mg/dL) | 1.20±0.07$^{a}$ | 1.01±0.04$^{a}$ | 1.13±0.08$^{a}$ | 0.90±0.18$^{a}$ | 1.05±0.04$^{a}$ | 0.93±0.05$^{a}$ |
| Serum unconjugated bilirubin (mg/dL) | 0.25±0.02$^{c}$ | 0.31±0.04$^{a}$ | 0.17±0.04$^{d}$ | 0.14±0.04$^{d}$ | 0.21±0.04$^{c}$ | 0.28±0.03$^{b}$ |
| Blood glucose (mmol/L) | 4.53±0.29$^{b}$ | 6.80±0.15$^{a}$ | 3.92±0.17$^{c}$ | 4.04±0.22$^{c}$ | 3.92±0.26$^{c}$ | 4.37±0.13$^{b}$ |

Values are mean ± SEM of five determinations. Means across the same row with different superscript letters are significantly different ($p<0.05$).

**Table 3** Selected kidney function indices of OV-treated rats administered VALEA

| | **Control (distilled water only)** | **Distilled water** | **Oestradiol valerate (2 mg/kg body weight)** | | | |
|---|---|---|---|---|---|---|
| | | | **Metformin(2.4 mg/kg body weight)** | ***V. album* extract (mg/kg body weight)** | | |
| | | | | **50** | **100** | **200** |
| Serum urea (mmol/L) | 3.86±0.34$^{a}$ | 3.66±0.26$^{b}$ | 3.72±0.36$^{b}$ | 2.82± 0.15$^{c}$ | 3.57±0.41$^{b}$ | 3.96±0.22$^{a}$ |
| Serum creatinine (µmol/L) | 88.34±3.86$^{b}$ | 83.54±3.73$^{b}$ | 87.06±3.42$^{b}$ | 99.05±1.05$^{a}$ | 96.08±1.66$^{a}$ | 94.69±2.31$^{a}$ |
| Serum uric acid(mmol/L) | 0.25±0.06$^{a}$ | 0.25±0.03$^{a}$ | 0.25±0.02$^{a}$ | 0.30±0.01$^{a}$ | 0.28±0.03$^{a}$ | 0.25±0.02$^{a}$ |
| Serum $Na^{+}$(mmol/L) | 136.00±2.12$^{b}$ | 142.00±1.14$^{a}$ | 130.20±1.39$^{d}$ | 136.80±1.39$^{b}$ | 129.40±1.60$^{d}$ | 134.20±0.86$^{c}$ |
| Serum $K^{+}$(mmol/L) | 3.64±0.25$^{c}$ | 3.54±0.11$^{d}$ | 3.46±0.30$^{d}$ | 3.50±0.11$^{d}$ | 4.16±0.05$^{b}$ | 4.64±0.22$^{a}$ |
| Serum $Cl^{-}$(mmol/L) | 96.40±0.75$^{a}$ | 97.00±1.48$^{a}$ | 95.20±1.39$^{a}$ | 94.10±2.08$^{a}$ | 89.100±1.40$^{b}$ | 88.00±1.38$^{b}$ |
| Serum $HCO_3^{-}$ (mmol/L) | 24.20±1.07$^{a}$ | 27.60±1.36$^{b}$ | 26.80±0.58$^{b}$ | 25.60±0.75$^{b}$ | 26.00±0.84$^{b}$ | 24.00±1.30$^{a}$ |

Values are mean ± SEM of five determinations. Means across the same row with different superscript letters are significantly different ($p<0.05$).

All the doses of VALEA and metformin had no significant ($p>0.05$) effect on the OV-induced increase in FFAs, as all were significantly ($p<0.05$) higher than the normal control value (Table 4). In contrast, metformin and VALEA at 100 mg/kg

bdwt lowered the OV-induced increase in triacylglycerols (Table 4). Total cholesterol values were unaffected by the treatments, but significant decrease by OV+metformin and significant increase by OV+200 mg/kg bdwt of VALEA were observed. VALEA at 100 and 200 mg/kg bdwt significantly reversed the OV-induced reduction in HDL. VALEA at 50 mg/kg bdwt had no significant effect on the OV-induced reduction, while metformin significantly ($p < 0.05$) led to further lowering of HDL (Table 4). OV alone had no significant effect on the LDL, while OV-treated rats given metformin and extracts (at 50 and 100 mg/kg bdwt) led to significantly reduced values and elevated value at 200 mg/kg bdwt of VALEA (Table 4). The extract at 50 mg/kg bdwt did not significantly affect the OV-induced increase in VLDL, while metformin and 100 mg/kg bdwt of VALEA significantly attenuated the increase. However, the VLDL level was further elevated by VALEA at 200 mg/kg bdwt. The OV-treated rats that received 100 mg/kg bdwt of VALEA had significantly lower value of the computed AI, when compared with the normal control value, while all the other treatments had no significant effect on the computed AI (Table 4).

**Table 4** Serum lipid profile of OV-treated rats administered VALEA

| **Serum lipids** | **Control (distilled water only)** | **Distilled water** | **Oestradiol valerate (2 mg/kg body weight)** | | | |
|---|---|---|---|---|---|---|
| | | | **Metformin(2.4 mg/kg body weight)** | ***V. album* extract (mg/kg body weight)** | | |
| | | | | **50** | **100** | **200** |
| Free fatty acids (mg/dl) | 0.62±0.05$^{a}$ | 0.77±0.03$^{b}$ | 0.72±0.03$^{b}$ | 0.75±0.03$^{b}$ | 0.74±0.02$^{b}$ | 0.74±0.08$^{b}$ |
| Triacylglycerols (mg/dl) | 124.56±2.96$^{ab}$ | 147.18±7.43$^{c}$ | 117.45±8.91$^{a}$ | 140.88±4.19$^{bc}$ | 132.91±7.58$^{b}$ | 156.78±5.78$^{c}$ |
| Total cholesterol (mg/dl) | 77.89±2.77$^{b}$ | 80.87±4.63$^{b}$ | 50.69±3.44$^{a}$ | 69.24±8.16$^{b}$ | 74.74±5.54$^{b}$ | 98.20±1.15$^{c}$ |
| High density lipoprotein (mg/dl) | 10.72±0.76$^{c}$ | 7.98±0.54$^{b}$ | 5.34±0.84$^{a}$ | 7.07±0.71$^{b}$ | 14.41±1.38$^{d}$ | 10.67±0.88$^{c}$ |
| Low density lipoprotein (mg/dl) | 42.05±2.715$^{c}$ | 42.65±4.13$^{c}$ | 21.86±3.63$^{a}$ | 33.99±7.19$^{b}$ | 33.75±5.60$^{b}$ | 56.17±1.52$^{d}$ |
| Very low-density lipoprotein (mg/dl) | 24.91±0.59$^{a}$ | 29.45±1.49$^{c}$ | 23.49±1.78$^{a}$ | 28.18±0.84$^{c}$ | 26. 58±1.52$^{b}$ | 31.35±1.16$^{d}$ |
| Atherogenic Index | 3.98±0.33$^{a}$ | 5.41±0.58$^{a}$ | 4.42±0.69$^{a}$ | 4.59±0.69$^{a}$ | 2.43±0.46$^{b}$ | 5.40±0.46$^{a}$ |

Values are mean ± SEM of five determinations. Means across the same row with different superscript letters are significantly different ($p<0.05$).

In addition, exposure of rats to OV resulted in altered liver morphology, characterized by fat globules or hepatic steatosis (Plate 1b), in contrast to the liver histoarchitecture of the control rats, having intact hepatocytes with their portal veins (Plate 1a). All doses of VALEA ameliorated the hepatic degeneration induced by OV treatment and hepatocytic portal veins were identical to the control (Plates1d-f) while the metformin caused mild inflammation, shown by presence of monocytes around the hepatic portal vein (Plate 1c). Moreover, OV, metformin and VALEA doses did not change the nephron morphology. The glomeruli, proximal and distal convoluted tubules were intact (Plates 2b-f) and comparable to the distilled water control (Plate 2a). Furthermore, the heart photomicrograph of the OV-treated rats showed myocarditis, indicated by zone of eosinophil infiltration within the myocardium (Plate 3b), whereas VALEA attenuated the inflammation, and the cardiomyocyte nuclei and the muscle striations (Plates 3d-2f) were similar to the control (Plate 3a) and metformin (Plate 3c) groups.

Sex hormones, their metabolites and analogues as well as herbal products can induce liver damage. In this study, OV was used to induce PCOS in female rats, with a view to accessing the potential ameliorating effects of VALEA.

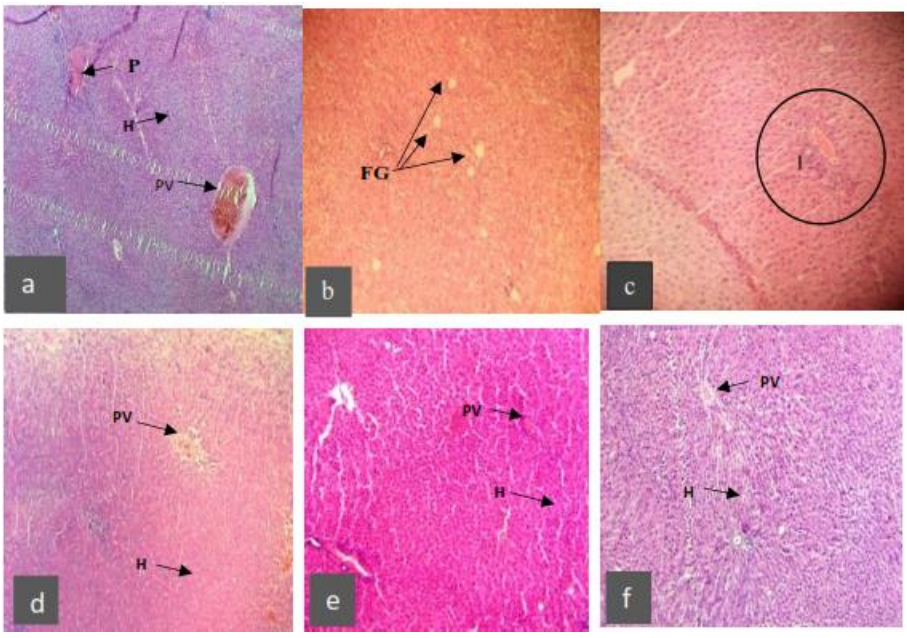


**Plate 1 (a)** Cross section of liver of distilled water control rat **(b)** Cross section of the liver of 2 mg/kg bdwt OV-treated rat administered distilled water **(c)** Cross section of the liver of OV-treated rat administered 2.4 mg/kg bdwt metformin **(d)** Cross section of the liver of OV-treated rat administered 50 mg/kg bdwt VALEA **(e)** Cross section of the liver of OV-treated rat administered 100 mg/kg bdwt VALEA **(f)** Cross section of the liver of OV-treated rat administered 200 mg/kg bdwt VALEA (X100, H & E stain, PV: portal vein, FG: fat globules (fatty) liver cells, H: hepatocytes, I: inflammation)

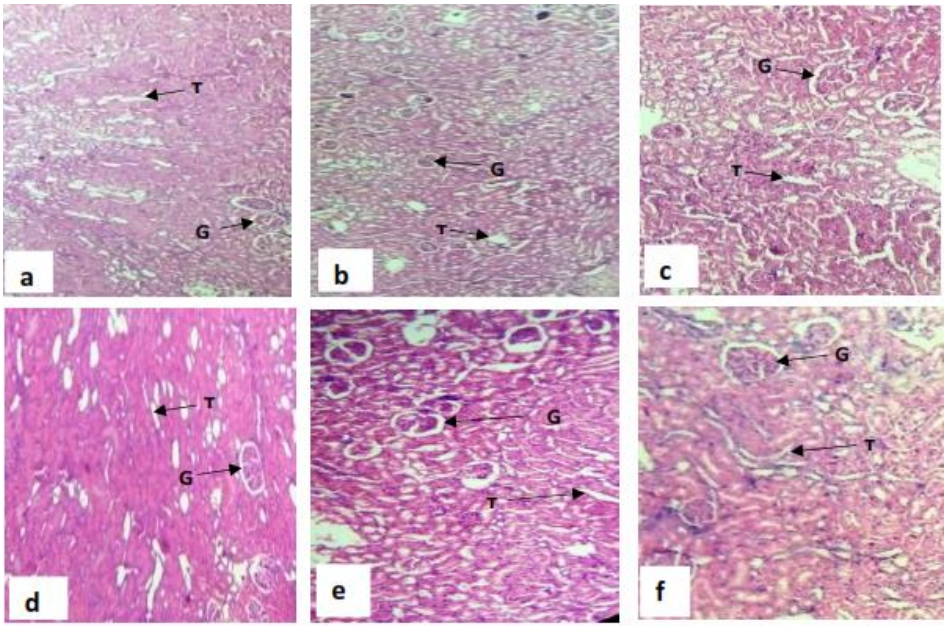


**Plate 2 (a)** Photomicrograph of the kidney of distilled water control rat **(b)** Photomicrograph of the kidney of 2 mg/kg bdwt OV-treated rat administered distilled water **(c)** Photomicrograph of the kidney of OV-treated rat administered 2.4 mg/kg bdwt of metformin **(d)** Photomicrograph of the kidney of OV-treated rat administered 50 mg/kg bdwt VALEA **(e)** Photomicrograph of the kidney of OV-treated rat administered 100 mg/kg bdwt VALEA **(f)** Photomicrograph of the kidney of OV-treated rat administered 200 mg/kg bdwt VALEA (X100; H&E stain, G: glomeruli; T: tubules)

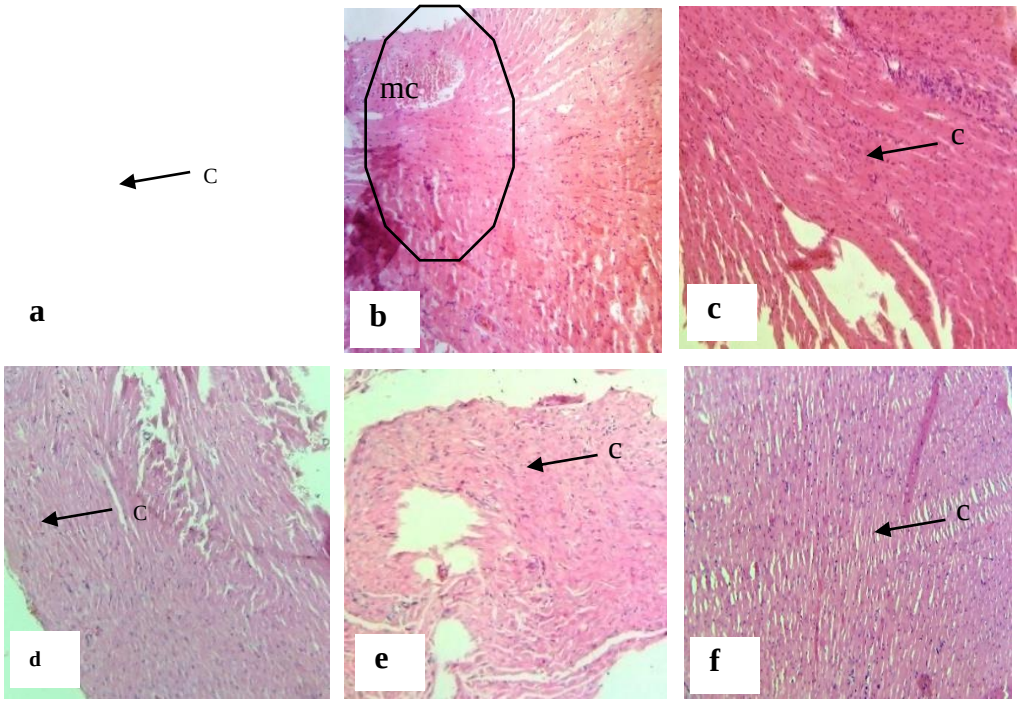


**Plate 3** Photomicrographs of hearts of OV- treated rats administered VALEA. MC: myocarditis (eosinophil infiltrate), C: cardiomyocytes. **(a)** Control (0.5 ml/kg bdwt distilled water); **(b)** OV+Distilled water (0.5 ml/kg bdwt); **(c)** OV+Metformin (2.4 mg/kg bdwt); **(d)** OV+ VALEA (50 mg/kg bdwt); **(e)** OV+ VALEA (100 mg/kg bdwt); **(f)** OV+ VALEA (200 mg/kg bdwt)

ALP is primarily found in the bone and liver, with traces in the kidney and intestine. An elevated ALP in the kidney, induced by OV, may indicate an activation of the kidney's functional activity and an increased kidney enzyme production [41] or influx of ALP from neighbouring tissues. This is corroborated by the reduced liver ALP seen in OV-treated rats which might indicate hepatocellular damage. Metformin administration might impair mitochondrial respiration and lactate metabolism in the liver and kidney [42]. The metformin associated lactic acidosis might cause organ injury and impaired function, which may explain the effect of metformin on the liver and kidney ALP. The 50 mg/kg bdwt of VALEA was most promising of all the doses as a potential attenuator of this effect. At 50 and 100 mg/kg bdwt, however, VALEA showed a synergistic impact by exacerbating the OV-induced decrease in liver ALP, showing that VALEA may also have an inactivating effect on liver ALP. The lower dose related variation in the VALEA extract metabolites in these tissues might account for the ameliorating effects on the ALP activity

Elevated serum AST and GGT of OV-treated rats may indicate alterations in cell membrane permeability, while elevated liver and serum ALT may indicate OV-stimulated *de novo* ALT biosynthesis. This may explain the increased extracellular presence of ALT in the serum; this leakage is also supported by decreased liver ALP, indicating loss of plasma membrane integrity. Attenuation by VALEA strongly indicates its hepato-protective potential as similarly reported against $CCl_4$-induced liver malfunction in rats [21].

Hexokinase, the first regulatory enzyme in the glycolytic pathway is both insulin-dependent and insulin-sensitive. Resistance to insulin causes defective glucose uptake in the peripheral tissues and accumulation of glucose 6-phosphate in plasma membranes [43]. This could, by negative feedback inhibition, decrease hexokinase activity. The decreased serum hexokinase activity of the OV-treated rats may therefore, be an indication for defective glucose uptake in the peripheral tissues and/or impaired cellular glucose oxidation. VALEA, at the highest dose, increased hexokinase activity towards the control, which implies enhanced glucose uptake and metabolism. This increased hexokinase activity by VALEA may also be connected with regulation of insulin secretion, improved insulin sensitivity and action, and anti-diabetic effects, cumulating in improved glucose metabolism by the plant as previously reported [16].

Albumin and globulin are the major soluble components of TP. Serum albumin concentration is an essential indicator of albumin turnover, therefore, the unchanged serum TP, albumin, and globulin in OV-treated rats administered VALEA suggests unaffected liver synthetic ability. This, however, contradicts the study of Adelakun *et al*. [44], which reported a decreased TP in letrozole-induced PCOS rats that was elevated by *Cyperus esculentus* tuber extract. The decrease in serum TBIL in both OV-and extract-treated animals is indicative of its decreased production in the liver, which may have resulted from impaired hepatic function or liver injury. The OV-induced increase in UBIL further supports this assertion. Bile duct

obstruction, leading to bile build-up in the liver may explain the observed accumulation of serum UBIL [45]. Reversed of OV-induced accumulation of UBIL by 100 mg/kg bdwt of VALEA implies its positive effect on bilirubin metabolism.

Impaired glucose uptake can cause hyperglycaemia and consequently, obesity [5]. Hyperglycaemia state observed in the OV-treated animals might induced oxidative stress [42] which can cause organ damage and impaired function including synthesis and or activity as evidenced from the increased kidney ALP. The ability of VALEA to reduce the OV-induced elevated blood glucose suggests that VALEA may ameliorate abnormal glucose uptake, indicating anti-hyperglycaemic effect. This may occur by improving insulin sensitivity, action and enhancing the insulin signalling pathways. A previous study reported anti-hyperglycaemic effect of *C. loniceroides,* a mistletoe specie, which inhibited α-amylase and α-glucosidase [15].

Urea reduction by OV may be indicative of an increased glomerular filtration rate and renal excretion mediated by oestradiol and a related renal malfunction [46,47]. Excessive loss from the body, which may exceed the rate of synthesis, may contribute to the lowered serum concentration; hepatocellular dysfunction may also cause decreased synthesis. Attenuation by 200 mg/kg bdwt of VALEA indicates its positive effect on urea metabolism and excretion. Elevation of creatinine levels by VALEA indicates that it may decrease glomerular filtration or increase tubular reabsorption of creatinine in OV-treated rats. These observations contradict a previous report which revealed increased serum urea in OV-treated rats and unaffected creatinine levels of the PCOS, metformin and *Chamomile* flower extract-treated groups [48].

Potassium supplementation has vasodilation effect, promotes muscle contraction, blood flow and thereby reducing the pressure against blood vessel walls [49]. Therefore, elevated $K^+$ and decreased $Na^+$ and $Cl^-$ levels in serum of extract-treated animals suggest that the 100 and 200 mg/kg bdwt doses might cause natriuresis, accompanied by salt and water excretion and then, $Na^+/Cl^-$ reduction in the body of the animals, leading to decrease in blood pressure in the renal arteries. This implies that VALEA might demonstrate antihypertensive effects to control blood pressure and hypertension in OV-induced PCOS condition. Pechter *et al.*[14] also reported a significant decrease in systolic blood pressure effect of extract from the plant in rats. The increase in the serum $K^+$ level may be due to a high potassium content earlier reported in the extract [18].

Increasing hepatic oestradiol metabolism can induce hyperandrogenaemia. Elevated free testosterone can enhance lipogenesis, deposition of triacylglycerol in the adipose tissues, accompanied by enhanced intracellular LDL receptor pathway, thereby causing elevation of LDL and high AI [6]. This may explain the elevated triacylglycerol and decreased HDL in the OV-treated animals. This therefore, implies that the OV-treated animals may, in a long term, be prone to cardiovascular diseases such as hypertension. The observed elevated triacylglycerol and decreased HDL concurs with a previous report of dyslipidaemia characterized by abnormal TC, triglycerides, HDL and LDL with decreased serum HDL in Chinese women with PCOS [50]. Reversal of the OV-induced alterations in the serum lipids by VALEA indicates that it can modulate lipid metabolism in OV-induced PCOS condition in rats. Specie of this plant has also been reported to regulate fat accumulation by inhibiting FA synthase and decreasing expression of adipogenesis activators [10]. However, increased triglycerides and total cholesterol levels accompanied 200 mg/kg, the highest dose of the extract which may contribute to visceral or peripheral obesity in long time [6], implies that prolonged administration of the extract at high doses may be associated with atherogenic risk in the animals. This, however, need to be validated in further studies.

Hepatic fatty infiltration (fatty liver) observed in the OV-treated group might have resulted from hyperlipidaemia emanating from the OV metabolism [22,23]. This vacuolar degeneration of the hepatocyte was likewise returned toward normalcy by VALEA, which implies that it may protect against fat-mediated hepatocellular damage and prevent hepatic steatosis. This effect may be related to inhibition of FA synthase and the modulation of lipoprotein metabolism previously reported by the plant [10].

The unaltered glomerular and tubular histoarchitecture in the OV-treated rats and VALEA shows that there were no discernible detrimental effects of treatment on the nephron. This agrees with previously reported protective potential of VALEA on chemically-induced nephrotoxicity and kidney dysfunction, and improved kidney morphology and function in nephrectomised rats [14,20]. A study, however, reported a marked deformity in kidney morphology of OV-treated rats [46].

Myocarditis is an inflammation of the cardiac muscle, which can cause cardiac dysfunction and lead to cardiovascular diseases. It is characterized by increased myocardial presence of immune cells such as monocytes, neutrophils, eosinophils and T-lymphocytes which are produced in response to inflammation [51,52]. Attenuation of the inflammatory response by the VALEA implies that it might improve cardiac muscle metabolic abnormalities in the OV-treated rats. It might modulate response to injury, tissue repair in the myocardium and promote the physiological inflammation [53], thereby producing the relatively normal cardiomyocytes.

The hepatocyte degradation mediated by OV administration was confirmed biochemically by the increased UBIL and liver-specific enzymes ALT, AST, and GGT, as well as the decreased urea. VALEA may provide protection and/or preservation of the liver and kidney functional capacities in OV-treated rats due to the attenuation of hepatic degeneration and some biochemical changes (53%), with the exception of the aggravated liver ALP, TBIL, and creatinine, which accounted for 15%, and the absence of detectable adverse structural effect on the nephron and some other biomarkers (32%). Further studies are recommended to investigate the mechanism(s) by which the plant could exert the hepato- and nephro-protective potentials against progressive injury and malfunctioning.

## 4. Conclusion

Although, the VALEA in this study attenuated some of the biochemical changes in the liver, kidney and heart as well as hepatocellular degeneration and cardiac muscle inflammation induced by OV-treatment, with no detectable distortion in the histoarchitecture of the nephron. This demonstrates that VALEA relatively exhibited protective potential and preserved the liver, kidney and heart functional capacities. Further studies are needed to identify the specific phytochemicals involved and their mechanism(s) of action. The dose related lipidemic, hepato and reno-toxic effects found in the study are recommended to be further validated and the extract should be administered orally with caution.

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## Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are openly available in [repository name e.g "figshare"] at http://doi.org/[doi].

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