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Safety and risk assessment of methanol extract of *Ocimum gratissimum* leaf on Wistar rats

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Abstract

Methanol extract of *Ocimum gratissimum* was investigated for its effects on Wistar rats using some kidney and liver functional indices as 'markers'. Twenty- eight (28) albino rats weighing 180-200 g was randomly assigned into four groups (A-D) of seven animals each. Animals in groups B-D was orally administered on daily basis with 0.5 ml of the extract corresponding to 50,100 and 200 mg/kg body weight of the extract respectively for 14 days while those in the control group received orally 0.5 ml of distilled water. Rats in all the groups were sacrificed 24 hours after the completion of their respective doses and serum, liver and kidney tissue samples were collected for biochemical parameters. The extract significantly ($p<0.05$) decreased serum, liver and kidney ALP, AST and ALT. The extract also significantly ($p<0.05$) reduced serum urea, uric acid and creatinine. Contrastingly, the extract significantly ($p < 0.05$) increase in serum albumin, globulin and total protein respectively when compared with the normal control at 50, 100 and 200 mg/kg body weight respectively. The effect of administration of the extract on serum Na^+, K^+, Cl^- and HCO_3^- ions shows a significant ($p<0.05$) decrease when compared with the normal control with the exception of an increase in K^+ ion concentration at 200 mg/kg bwt when compared with the normal control. The biochemical alterations from this result suggest that the extract of *Ocimum gratissimum* may induce hyponatremia and hypokalaemia at high dosage possibly by altering the Na + /H – exchanger with aldosterone without causing assault or injury to the hepatic, nephrotic and /or tubular function.

Keywords: Aldosterone; Hepato cellular; Hyponatremia; Hypokalemia; *Ocimum gratissimum*; Toxicity

1. Introduction

From the very beginning of human existence man has familiarized himself with plants and used them in a variety of ways through the ages. Primitive man in search of food and to cope successfully with human sufferings began to distinguish those plants suitable for medicinal purpose from others with definitive pharmacological action, the growth of knowledge to cure diseases continued at an accelerating pace and the number of new plant-derived drugs increased likewise [1].

However, all plants synthesize phytochemicals which are beneficial for our health as they cannot be synthesized in the human body [2]. In traditional methods plants materials are tested for pharmacological purpose, if any evidence of activity is observed, the extract is fractioned and the active compound is isolated and identified [3]. Some of the

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chemotherapies such as anticancer, antihypertensive and anti migraine medications produced from natural products [4].

In folk medicine, a single herb formula may contain many phytochemical constituents such as alkaloids, terpenoids, flavonoids etc [1]. These chemical constituents function alone or in conjunction with one another to produce the desired pharmacological effects [5].

Plants synthesize both primary and secondary metabolite, the secondary metabolites includes alkaloids, flavonoids, saponins, terpenoids, steroids glycosides, tannins, volatiles oils etc [1]. The therapeutic efficacy of plants is because of these secondary metabolites potentials for curing diseases, this includes alkaloids have an antispasmodics, antimalarial analgesic, diuretic activities. Terpeniods are known for their antiviral anthelmintic, antibacterial, anticancer, antimalarial, ant-inflammatory properties; Glycosides are reported for antifungal and antibacterial properties; phenols and flavonoids have an antioxidant, anti-allergic, antibacterial properties and saponin are reported to have anti-inflammatory, antiviral, plant defense activities [3].

Ocimum gratissimum is one of such herbal medicinal plant whose phytochemical study shows the presences of several bioactive compounds [6] *O. gratissimum* is known as clove basil or lemon basil, Commonly known as "scent leaf" *Ocimum gratissimum* is a polymorphic branched, aromatic shrub, belonging to family *Lamiaceae*, as spice in food, it's a shrub that prefers moist and fertile soil during growth but tolerate drought after flowering [7].*O. gratissimum (lamiaceae)* also known as In Nigeria, it is '*efinrin'*(Yoruba), *diadoyal*(Hausa),*nchuanwu*(Igbo) [8].

In Nigeria, the plant grows virtually in all regions. It could be found in many farms, residential and industrial areas, it grows and survive well in south west Nigeria and could be found at backyards were it is not intentionally planted [9]. *O. gratissimum* has been extensively used in the traditional system of medicine in many countries, in the northeast of Brazil; it is used for medicinal, condiment and culinary purposes. The flowers and leaves of this plant are rich in essential oils, and so it is used in preparation of teas and infusion [10]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea [11]. The plant is used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, diseases of the eyes and skin, pneumonia, cough, fever and conjunctivitis [12].

Figure 1 *O. gratissimum* leaves

The shrub has been used for various purposes and leaves are more of significances, however, whole plant or even the seed also has ethno-pharmacological importance as they yield essential oil of economic importance [7].This present research determines the effect of extract of methanol *O. gratissimum* on liver and renal function indices of Wistar rats.

2. Material and methods

2.1. Plant materials

Fresh leaves of *O. gratissimum* were collected from the Federal Secretariat Farms, Calabar,Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 201 has been deposited for future reference at the Department's Herbarium.

2.2. Experimental animals

The albino Wistar rats were obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals were allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29 °C and 70 % respectively with 12 hours natural light-dark cycle. They were allowed food and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages.

2.3. Preparation of methanol extract of *O. gratissimum* leaf

The leaves of *O. gratissimum* were collected and dried at room temperature for a period of 21days until constant weight was obtained. The dried leaves were then pulverized to powdered form by a machine blender and sieved. Thereafter, 400 g of the pulverized plant material (*O. gratissimum*) was dissolved in 1200 ml of 70% methanol for 72 hours. This was followed with vacuum filtration and extracts was concentrated using an evaporator water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

2.4. Animal grouping and administration of extract

Twenty-eight (28) male Wistar albino rats were randomly picked and placed into plastic cages labeled A-D. Group A served as the control group while groups B-D were the test groups. The animals in group A were administered with distilled water orally. Group B were administered 50 mg/ kg body weight of methanol extract of *O. gratissimum*, group C were administered with 100mg/kg bodyweight while group D were administered with 200mg/body of methanol extract of *O. gratissimum* for 14 days.

2.5. Preparation of tissue homogenate

The liver and kidneys of the rats were removed under the same condition and the surrounding fatty tissues were removed from the organs, as they could make the homogenization process more difficult.

The process was carried out by blending each organ of each rat separately in 2mls of 1% glucose solution until a relatively smooth homogenate was formed. The homogenate of each organ was centrifuge for 15mins followed by extraction of the liquid homogenate into a sterile plane test tube.

2.6. Biochemical assay

At the end of the 14-day experimental period, the anesthesia was performed on all experimental animals. The anesthetized animals were bled by cardiac puncture. The blood samples were collected and centrifuged at a speed of 3000 rpm for 15 minutes and serum collected into plain sample bottles for biochemical analysis

2.7. Statistical analysis

Data were presented as a mean ± SD of five determinations. Statistical analysis was carried out using one way analysis of variance (ANOVA). Difference were statistically signify cant at $P<0.05$.

3. Results

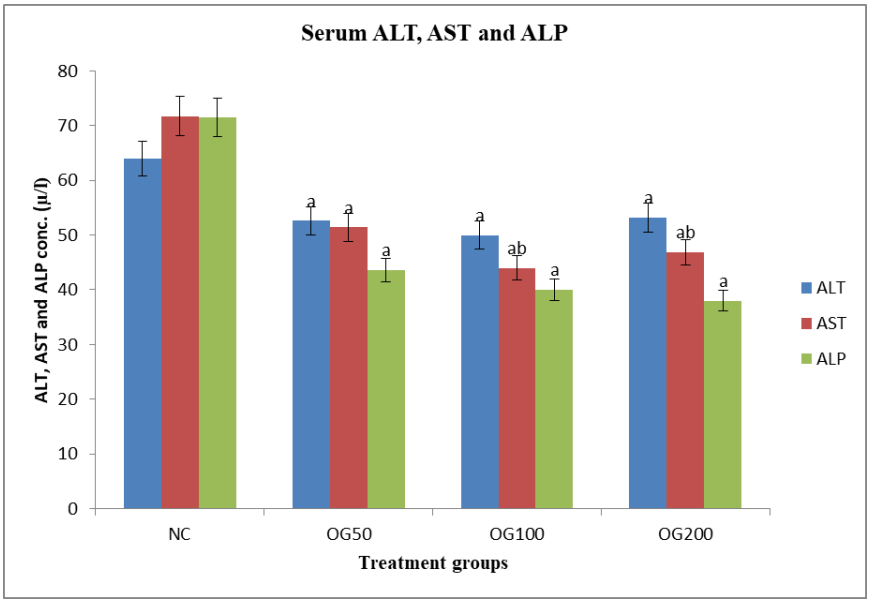
The results below depict the effect of administration of methanol extract of *O. gratissimum* on some biochemical parameters of Wistar rats. The result shows that the extract produced a significant ($p<0.05$) reduction on serum, liver and kidney ALT, AST and ALP when compared with the normal control (fig1-3).

The extract of *O. gratissimum* on serum urea and uric acid concentration also produced a significant ($p<0.05$) decrease on serum urea and uric acid concentration following the administration of 50, 100 and 200mg/Kg bwt when compared with normal control (fig 4).

Alternatively, the extract produced a significant increase in serum creatinine following the administration of the extract at 50 and 200 mg/kg bwt respectively when compared with normal control (Fig 5). The effect of the extract on serum sodium ion concentration revealed a significant (*p<0.05*) decrease on all the treated groups when compared with the control (fig 6).

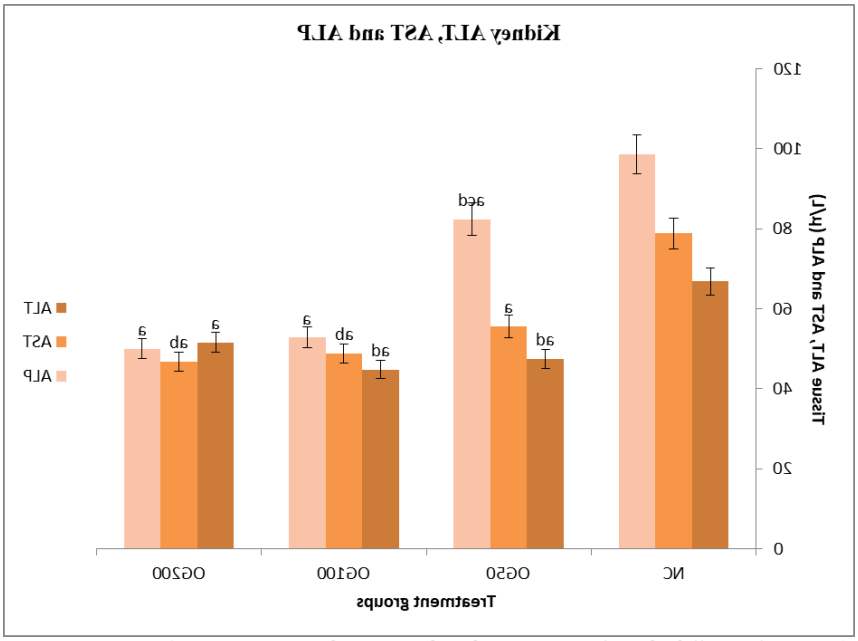
The effect of administration of the extract on K^+, Cl^- and HCO_3^- produced a significant (*p<0.05*) decrease at 50 and 200mg/kg bwt when compared with the normal control with the exception of an increase in K ion concentration at 200mg/kg bwt when compared with the normal control(fig 7).

The results below also revealed that the extract produced a significant increase (*p < 0.05*)in serum albumin, globulin and total protein respectively when compared with the normal control at 50, 100 and 200mg/kg body weight respectively (fig 8).



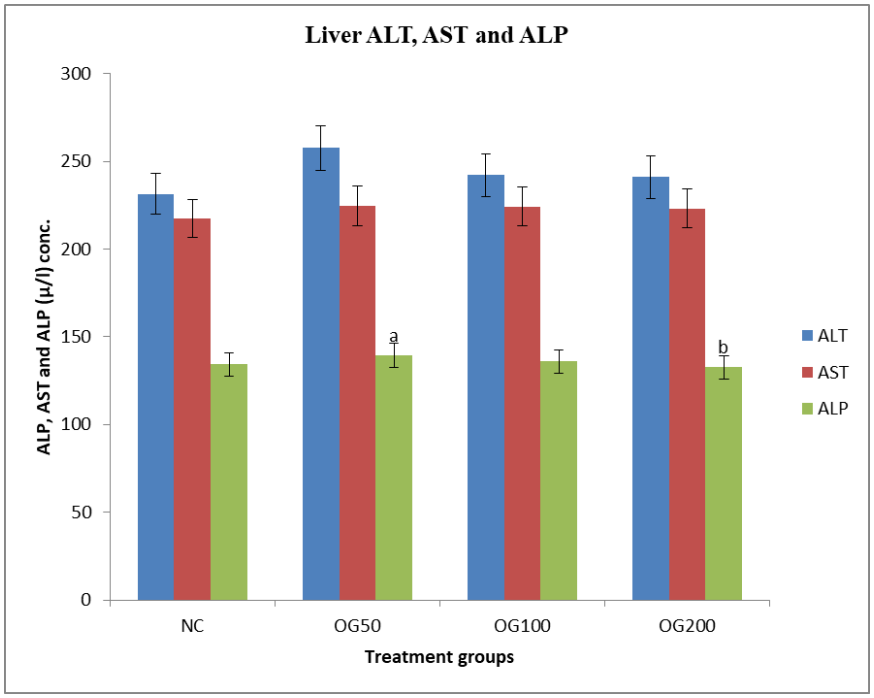
Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars; a = significantly different from NC (*P<0.05*), b = significantly different from OG_{50} (*P<0.05*), c = significantly different from OG_{100} (*P<0.05*) and d = significantly different from OG_{200} (*P<0.05*) Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 2 Effect of administration of methanol leaf extract of *O. gratissimum* on serum alanine aminotransferase, aspartate transaminase and alkaline phosphatase activities of Wistar albino rats



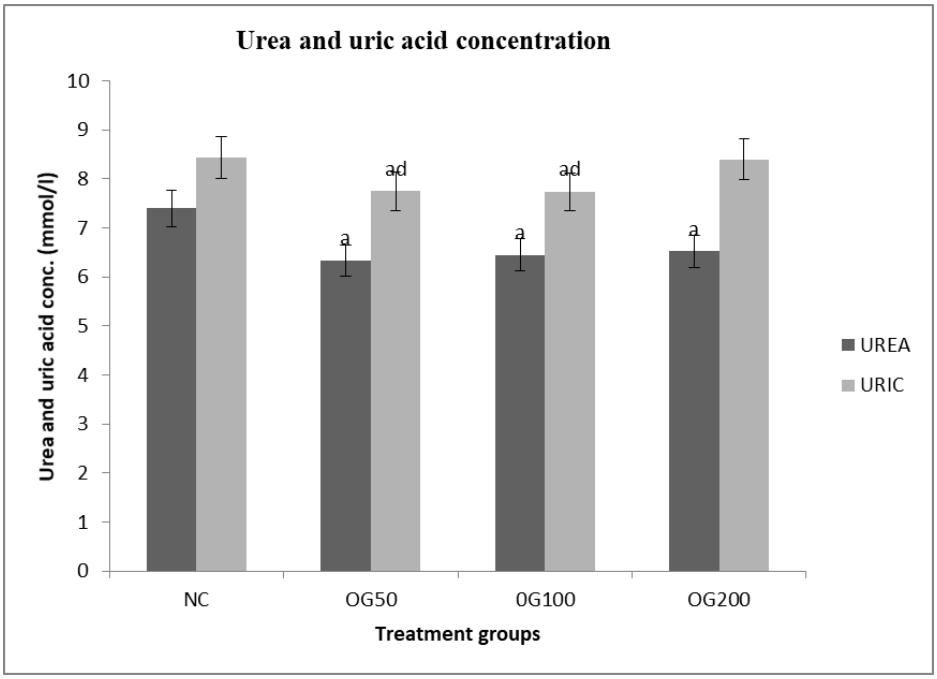
Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars; a = significantly different from NC (*P<0.05*); b = significantly different from OG_{50} (*P<0.05*); c = significantly different from OG_{100} (*P<0.05*) and d = significantly different from OG_{200} (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 3 Effect of administration of methanol extract of *O. gratissimum* on kidney alanine amino transferase, aspartate amino transferase and alkaline phosphatase of Wistar rats



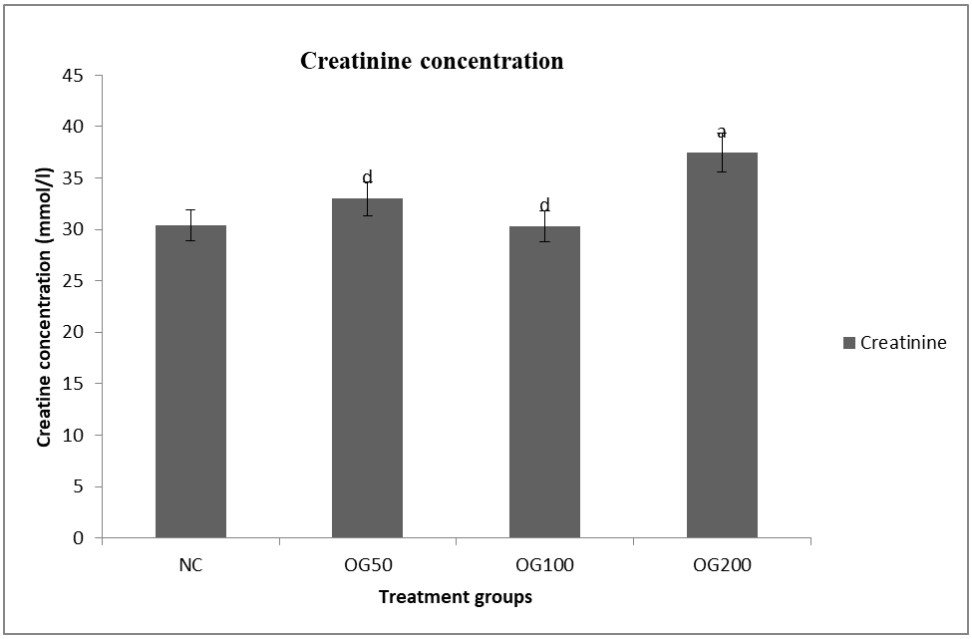
Values are expressed as mean ± SEM; n = 5 rats per group. The same colour bars; a = significantly different from NC (*P<0.05*) and b = significantly different from OG_{50} (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 4 Effect of administration of methanol extract of *O. gratissimum* on tissue (liver) alanine aminotransferase, aspartate transaminase and alkaline phosphatase activities of Wistar rats



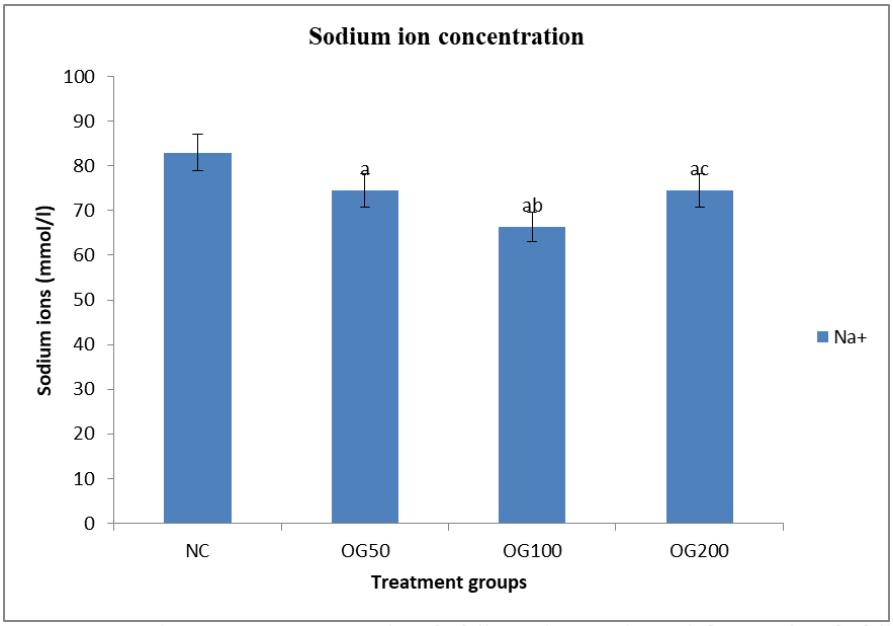
Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars, a = significantly different from NC (*P<0.05*) and d = significantly different from OG_{200} (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 5 Effect of administration of methanol extract of *O. gratissimum* on urea and uric acid concentration of Wistar rats



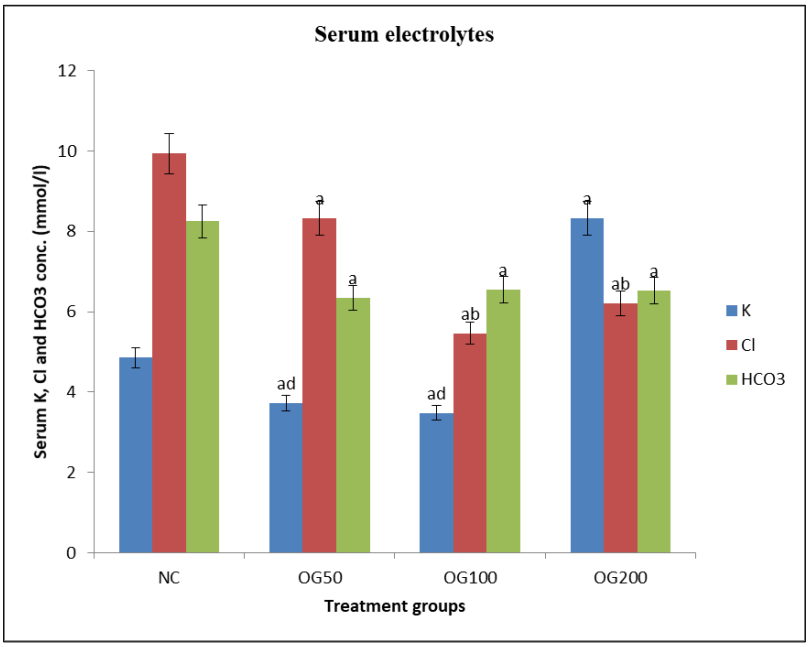
Values are expressed as mean ± STD; n = 5 rats per group. a = significantly different from NC (*P<0.05*) and d = significantly different from OG_{200} (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 6 Effect of administration of methanol extract of *O. gratissimum* on creatinine concentration of Wistar rats



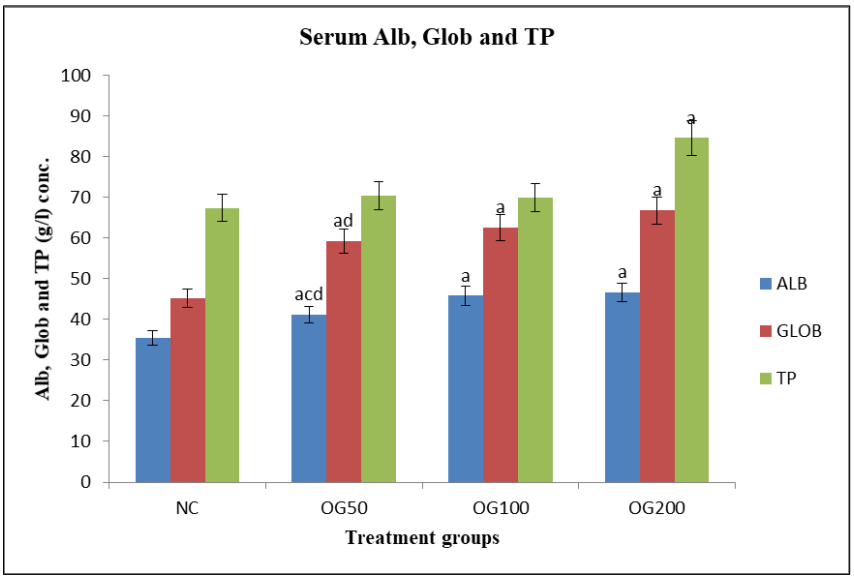
Values are expressed as mean ± STD; n = 5 rats per group. a = significantly different from NC (*P<0.05*); b = significantly different from OG_{50} (*P<0.05*) and c = significantly different from OG_{100} (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 7 Effect of administration of methanol extract of *O. gratissimum* on serum sodium concentration of Wistar rats



Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars; a = significantly different from NC (*P<0.05*); b = significantly different from OG_{50} (*P<0.05*); c = significantly different from OG_{100} (*P<0.05*) and d = significantly different from OG_{200} (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 8 Effect of administration of methanol extract of *O. gratissimum* on serum potassium, chloride and bicarbonate concentration of Witar rats



Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars; a = significantly different from NC ($p<0.05$), c = significantly different from OG_{100} ($p<0.05$) and d = significantly different from OG_{200} ($p<0.05$). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

Figure 9 Effect of administration of methanol leaf extract of *O.gratissimum* on serum albumin, globulin and total protein concentration of Wistar rats

4. Discussion

The assessment of serum enzymes, albumin , globulin , creatinine, urea, uric acid and electrolyte can be a reliable means of assessing the functional integrity of both the liver and kidney [13].

An electrolyte is any substance containing free ions that make the substance electrically conductive. Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids due to their ability to dissociate readily into their constituents ions or radicals, they compromise the single most important factor in the transfer and movement of water and electrolyte: between three divisions of extracellular and intracellular components [14].

Physiologically, the primary ions in electrolytes include sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), hydrogen phosphate (HPO_4^{2-}), and hydrogen carbonate (HCO_3^-) which have very diverse functions including: signal transduction, muscular contraction, maintenance of osmolality and fluid balance, nervous transmission, blood clotting, and enzyme function etc [15].They are found in blood, urine and body fluids and can be gotten from foods eaten and fluids drunk [16].Levels of electrolytes in the body can become too low or too high and particular subjected to the changes of the water levels in the body. Some causes of these imbalances include drugs, malnutrition, vomiting, diarrhea, sweating, or kidney problems.

From this present study, significant decrease in serum sodium ion concentration following oral administration of the methanol leaf extract may be due to excessive loss of heat from the body fluid. It may also be attributed to decreased production of aldosterone to other mineral corticoids which will in turns decrease the reabsorption of sodium ion concentration. Aldosterone can achieve this due to its action on the membrane, aldosterone receptors has been linked to stimulating Na + /H – exchanger. It also suggests that the extract may triggers hyponatremia, possibly due to assault on the functional integrity of the kidney.

The increased level of serum K^+ following the administration of the plant extract at doses 200mg/kg body weight suggest that at high dosage above 100mg/kg body weight the extract may induce hyperkalaemia which may alter the functional integrity of the kidney. Also, the significant increase in serum potassium concentration observed at 200mg/kg body weight in this study suggests a possible alteration on the sodium pump that maintains the constant of the extracellular potassium.

Serum chloride and bicarbonate ions are group of electrolytes that can be used to asses renal functions therefore, the significant decrease in both serum chloride and bicarbonate ions at various doses may be an indication of no tubular or

glomerular dysfunction. It appears that the extract might not induce any pathological condition that might result in impairment on renal function.

Serum urea, uric acids and creatinine levels are important biomarkers as they play a pivotal role in diagnosis and follow-up of kidney failure. Urea, one of the by-products of protein metabolism, accumulates in the blood of patients with kidney failure and causes uremia [17]. Urea nitrogen is a normal waste nitrogen product found in blood that comes from the breakdown of protein from foods. Healthy kidneys remove urea nitrogen from blood, but the level of urea in blood rises with kidney failure. Urea is the major nitrogen-containing product of protein catabolism while uric acid is the major product of the catabolism of purine nucleotides, however, the bulk of purine are ultimately excreted as uric acid from degradation of endogenous nucleic acids. The decreased levels in serum urea concentration following the administration extract of *O.gratissimum* suggest that the extract may have altered the protein catabolism.

Uric acid is the major product of the catabolism of purine nucleotides, however, the bulk of purine are ultimately excreted as uric acid from degradation of endogenous nucleic acids. Elevation of serum uric acid levels have been implicated in various disorders including gout, increased nuclear breakdown and renal diseases possibly by inhibiting pyrimidine nucleotide formation and cellular growth [18].The significant reduction in serum uric acid following the administration of the extract is an indication that the extract may not impair the functional integrity of the kidney.

However, urea and uric acid levels alone cannot be used as determinants of kidney function as they are affected by quite a number of factors. Other significant biomarker of the kidney is creatinine. It is produced from muscles and excreted through the kidneys along with other waste products. Creatinine concentration in serum is maintained by the balance between its generation and excretion by the kidneys. It has been estimated that 2% of the body's creatine is converted into creatinine every day, resulting in the daily generation of creatinine at a fairly constant rate (male: 20 to 25 mg/kg/day; female 15 to 20 mg/kg/day) [19]. Males have higher serum creatinine levels than females because males have greater muscle mass. The quantity of creatinine in serum depends on their generation, glomerular filtration and tubular secretion of serum creatinine. Therefore the creatinine concentration, considered a significant marker in renal dysfunction was not altered except at 50 and 100mg/kg body weight but might alters the tubular function at 200mg/kg bodyweight or higher dosage.

Alkaline Phosphatase (ALP) is another biomarker enzyme for assessing the integrity of plasma membrane [20]. Increase in the activities of alkaline phosphatase is an indication that there could be damage due to cytotoxic effect of a drug thereby resulting to leakage of this enzyme from the liver/kidney into the serum. Such increase in alkaline phosphatase activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital processes since there may be indiscriminate hydrolysis of phosphate esters in the tissue [21]. Increased activity of ALP which occurs due to de novo synthesis by kidney/ liver cells is a reliable marker of nephrotic/hepatobiliar dysfunction due to assaults [22]. Contrastingly, the decrease in serum, liver and kidney ALP following the administration of methanol extract of *O.gratissimum* suggests that the extract may not cause injury or assault to the functional integrity of the kidney and the liver.

Alanine aminotransferase (ALT) enzymes are sensitive biomarkers of hepatic and renal status [23]. Although ALT and AST are synthesized in the liver, they are also present in serum and in various tissues like kidney. In particular, ALT serum levels become elevated during liver/renal diseases, and therefore, it is considered a more specific marker for kidney/ liver injury than AST [23]. The observed decrease in both kidney and serum ALT is an indication that the extract may contain some metabolites which may protect the kidney function . Aspartate aminotransferase (AST) is primarily found in the liver mitochondrial and cytoplasm, it is also found in heart, muscle, kidney and brain. Its serum level increases in hepatic/nephrotic necrosis, myocardial infarction and muscle injury [24]. In the present work, the significant decrease observed in the activities of liver, kidney and serum AST may suggest that the extract may not induce any assault or alteration on hepatic and tubular or nephrotic functions.

The liver is also the sole site for the synthesis of albumin, which makes up approximately 60% of serum protein concentration [25]. More so, the concentrations of albumin, globulin and total protein in the serum can be used to assess the health status of the liver and can also be used to ascertain different type of liver damage [26]. The significant increase in the concentration of serum albumin, globulin and total protein observed following the administration of the extract may indicate that the extract produced no cellular toxicity of the extracts on the liver .It also suggest that it pose no threat to the functional integrity of the liver.

5. Conclusion

The biochemical alterations from this result suggest that the extract of *Ocimum gratissimum* may induce hyponatremia or hypokalaemia at high dosage possibly by altering the Na + /H – exchanger without causing assault or injury to the integrity of both the hepatic and nephrotic or tubular function.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have not declared any conflict of interests.

Statement of ethical approval

Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of University of Cross River State, Calabar, Nigeria (approval number FBMS/CRUTECH/15/022).

References

[1] Arvind K.S (2016). Medicinal plants: future source of new drugs. International Journal of Herbal Medicine.4(4): 59-64

[2] Martinez M J A , Lazaro R.M, del Olmo, L.M.B and Benito, P.B (2008).Anti-infectious activity in the anthemideae tribe. In: Atta-ur- (Ed.) Studies in Natural Products Chemistry.35:45-516.

[3] Jamshidi-Kia F, Lorigooini Z, & Amini-Khoei, H (2018).Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology, 7(1):11-18.

[4] Joo YE. (2014). Natural product-derived drugs for the treatment of inflammatory bowel diseases. Intestinal Research.12 :103–109.

[5] Parasuraman, S., Thing, G. S., & Dhanaraj, S. A. (2014). Polyherbal formulation: Concept of ayurveda. Pharmacognosy reviews, 8(16), 73.

[6] Pandey S, Shukla A, Pandey S, Pandey A (2017).An overview of resurrecting herb 'Sanjeevani' (Selaginella bryopteris) and its pharmacological and ethnomedicinal uses. The Pharmacological Innovation .6:11-14.

[7] Olaleye R.O, Akomolafe RO, Imafidon EC, Ogundipe DJ, Olukiran S O and Oladele A A(2016). Treatment with methanolic extract of Ocimum gratissimum (Linn.) leaf reversibly normalizes urine protein-creatinine ratio in Wistar rat model of gentamicin-induced kidney injury. International Journal of Medicine and Biomedical Research. 5(3); 156-171

[8] Ajiboye, A.A., Fadimu, O.Y., Ajiboye, M.D., Agboola, D.A., Adelaja, A.B and Bem, A.A.,2014. Phytochemical and Nutritional Constituents of Some Common Vegetables in South-West, Nigeria. Global Journal Science Frontier Research .14:1-7.

[9] Ekwenchi M M, Oluigbo J and Akpuaka A (2014). Antibacterial activity of n-hexane extract of Ocimum gratissimum leaves. IOSR Journal of Applied Chemistry.7:6-10.

[10] Rabelo M, Souza E.P, Soares P M G (2003). Antinociceptive properties of the essential oil of Ocimum gratissimum L. (Labiatae) inmice. Brazilian Journal of Medical and Biological Research ,36: 521-524

[11] Ekpo K. E, Anosike E. O, Ikeh C, Uwakwe A and AAhroghro E. M (2012).Curative potential of aqueous extract of scent leaves (Ocimum gratissimum) on cisplatin induced hepatotoxicity on albino Wistar rats. Journal of Pharmaceutical and Scientific Innovation.1(4):1-8

[12] Adewale A Y, Adeshina I & Yusuf O Y (2017). Anesthetic effect of Ocimum gratissimum extract on Oreochromis niloticus juveniles. Eur Exp Biol, 7:2–7

[13] Al-Mamary M, Al-Habori M, Al-Aghbari A M, Baker MM (2002). Investigation into the toxicological effects of Catha edulis leaves: A short-term study in animals. Phytotherapy Research.16:127-132.

[14] Dasofunjo K,Nwodo OFC,Yakubu O E,Ejoba R,Ukpanukpong R.U,Ipav S,Ugwu M N,Okafor AI and Ezugwu H.C (2013).Toxicological implication of Piliostigma thonningii leaves on male Wistar albino rats. European Journal of Experimental Biology 3(3):652-655

[15] Dasofunjo K, Okwari, O O, Obembe AO,Olatunji T L ,Ezugwu, HC and Osim E E (2014) .Assessment of the effect of Cannabis sativa and Nicotiana tobacum leaves onsome haematological and liver function indices of albino ratsIOSR Journal of Pharmacy and Biological Sciences (IOSRJPBS) . 9 (Issue 1 Ver. II) : 33-40.

[16] Dasofunjo K, Asuk A ,Okwari O O and Oli M (2016).Haematological and kidney function indices of Piliostigma thonningii leaf extract administration following pefloxacin induced toxicity in Wistar rats.British Journal of Medicine and Medical Research 16(11):1-8.

[17] Entedhar RS and Nawal A M (2016). Biochemical changes in chronic renal failure pre and post haemo dialysis. Journal of Environmental Science Engineering Technology .5:190-195.

[18] Dasofunjo K, Berinyuy EB, Nkanu E E ,Mba CE, Ujong PU , Ati B Uand Monjok E N (2018) Maternal changes on histological and liver function indices exposed to Piliostigma thonningii extract following acetaminophen toxicity in pregnant rats International Journal of Innovative Research and Review 6(4) :1-11

[19] Kalantar-Zadeh K, Shah A, Duong U, Hechter RC, Dukkipati R and Kovesdy C P (2010). Kidney bone disease and mortality in CKD: revisiting the role of vitamin D, calcimimetics, alkaline phosphatase, and minerals. Kidney International. 78(S117):S10.

[20] Suresh M, Mallikarjuna R N and Sharan BSM(2012). Haematological changes in chronic renal failure. International Journal of Scientific and Research Publications; 2:2250-2253.

[21] Yakubu MT, Adesokan A A and Akanji M A (2006).Biochemical change in the liver kidney and serum of rat following chronic administration of cimetidine. African Journal of Biomedical Research.9:213-218.

[22] Muriel P and Escobar Y (2003) Kupffer cells are responsible for liver cirrhosis induced by carbon tetrachloride. Journal Applied Toxicology.23(2):103-108.

[23] Dasofunjo K, Nwodo OFC, Ipav SS and Barminas Z l (2012). Effect of the extract of Pliostigma thonningii leaves on kidney function indices and haematological parameters of male albino Wistar rats J.Nat.Prod.Plant.Resour.,2(6):670-674

[24] Srinivasan, K and Krishnamurthy, R (1997).Effects of β and γ isomers of hexachlorocyclohexane on some liver and kidney enzymes in albino rats. Current Science.46 (17):598-600.