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HISTOLOGICAL AND BIOCHEMICAL EFFECTS OF AQUEOUS EXTRACT OF OCIMUM GRATISSIMUM ON THE LIVER AND KIDNEY OF ADULT WISTAR RATS

[1]Ebeye OA, [2]Ekundina OV, [1]Wilkie IE

1. Department of Anatomy and Cell Biology, Faculty of Basic Medical Science Delta State University, Abraka, Delta State, Nigeria.
2. Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria

Corresponding Author: Ekundina OV
Email:Kemvic30@gmail.com

ABSTRACT

Aim: Ocimum gratissimum is used commonly in traditional medicine to treat certain diseases. This study accessed the histological and biochemical effects of aqueous extract of Ocimum gratissimum leaves on the kidney and liver of adult Wistar rats.

Methods: Twenty four adult Wistar rats were assigned into four groups (A, B, C and D) of 6 rats each. Group A was used as control, and B, C, and D were the treatment groups. Each treatment group received 300, 400 and 500mg/kg respectively of aqueous extract of Ocimum gratissimum orally for twenty one days. The animals were sacrificed on the 21st day by cervical dislocation. Blood was collected by cardiac puncture for biochemical analysis. The liver and the kidney were collected, fixed in 10% formal saline and processed for light microscopy.

Results: Rat weight showed no significant difference between control and treatment group. Biochemical analysis showed very mild decrease in serum ALP, ALT and AST levels when compared with the control. There was no significant difference in serum creatinine and urea levels when compared with the control. The histological study of the liver revealed peripotal inflammatory cell infiltrates and central vein vascular congestion. Histological study of the kidney showed mild to severe intestitial infiltration by inflammatory cells and increased vascular congestion.

Conclusion: Ocimum gratissimum may be useful in culinary dishes and also in the treatment of certain ailments but systemic toxicity is also possible and this is dose dependent. Caution should therefore be exercised in the therapeutic use of the plant.

Keywords: Ocimium gratissimum, Liver, Kidney, Histology, Biochemical

INTRODUCTION

Plants food especially vegetables contribute substantially to both local and ethno medicines in developing countries especially Nigeria Okafor, (1980), Gbile and Adesina, (1989). Recently the exploitation of wild plants for medicinal purposes has gained more acceptances in many countries of the world. To further underscore the importance of herbal medicine, most national

governments have established the traditional medicine regulatory council under the supervision of their various health ministries to tap the numerous potentials of herbs. This may be because traditional medicine has long been practiced even before the orthodox medical practice came into existence (Okafor, et al., 1980). The plant being investigated is popularly called African basil; scientifically it is called Ocimum gratissimum. It is a shrub commonly found in gardens and around village huts (Iwu, 1993). It is used commonly in traditional medicine to treat diseases such as upper tract infection, diarrhea, headache, pneumonia and also in the treatment of cough, fever and conjunctivitis (Correa, 1932; Onajobi, 1986). Ocimum gratissimum belongs to the family laminacea and is widely distributed in the tropical and warm regions of the world (Okigbo and Ogbonaya, 2006). Leaves of Ocimum gratissimum have been found to contain methylchaylcol, linalool, eugenol, thymol, and xanthamicrol and the amount produced is dependent on the area that is cultivated as well as part of the plant (Okujagu, et al., 2005; Odebiyi and Sofowora, 1978).

Ocimum gratissimum has both culinary and medicinal uses (Iwu, 1986). It is used mainly to flavor food and meat (Okigbo, 1977). The component of Ocimum gratissimum has biological activities such as antidiabetic, antiseptic, antitussive, antihelmitic antiplasmodic, antimicrobial, hepatoprotective and nephroprotective properties (Gbolade, 2009; Akinyemi, et al., 2004, Eboh and Ekundina (2012). Ocimum gratissimum also known as clove Basil, African Basil, and in Hawaii as wild Basil, is a specie of Ocimum. Ocimum gratissimum is known by various names in different parts of the world. In Nigeria, it has different names; in Yoruba language, it is known as Efiarinajase; Nchu-nwu in Igbo, in Benin language, it is known as Ebavbokho, Aaidoya ta gida in Hausa, Nchonwu in Igbo. (Iwu, 1993). Ocimum gratissimum has been used extensively in traditional system of medicine in many countries. In the north-east of Brasil, the flowers and leaves of the plant are rich in essential oils so it is used in the preparation of tea and infusion (Rabelo, et al., 2003). In the coaster areas of Nigeria, the plant is used in the treatment of Epilepsy, high fever and diarrhea (Effraim et al., 2003). In the savannah areas, decoctions of the leaves are used to treat mental illness (Akinmoladun, 2007). Ocimum gratissimum is used by the Igbo community of south eastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh (Ijeh, et al., 2005).

MATERIALS AND METHOD

Twenty four adult Wistar rats weighing between 130-190g were used for the study. The animals were kept in the animal house of the Faculty of Basic Medical Sciences, College of Health Science, Delta State University, Nigeria. They were allowed to acclimatize for a period of two weeks and were feed with growers mash and tap water. Fresh leaves of Ocimum gratissimum plant were first separated from the stalk, rinsed with water to remove dirt, air dried at room temperature and milled to fine powder. The extract was prepared by the use of a substrate extractor at a temperature of $35^0C - 40^0C$. The rotary evaporator was then used to concentrate the extract to 10% of its original volume at a temperature of 40^0C. The concentration was put in an oven for complete dryness. 5g of the extract was dissolved in 100ml of distilled water. The rats were randomly grouped into four of six animals per group. Group A was used as the control, Groups B, C and D received the aqueous extract of Ocimum gratissimum at doses 300mg/kg, 400mg/kg, 500mg/kg respectively. Administration of extract was carried out using the oral route by means of 2ml calibrated syringe with attached oral cannula so that actual dose can be determined and injected into the animal. The animals received their doses everyday for a period of twenty one (21) days. The rats were sacrificed twenty one days after extract administration using cervical dislocation and an incision made on the midline of the ventral surface of the Wistar rats and the kidney and liver excised. Blood was gotten from the animal by cardiac puncture. In each sacrifice, the kidney and liver were dissected out and put into labeled specimen bottles which contained 10% formal saline for further tissues processing.

Biochemical Analysis: The activities of urea, creatinine, Alkaline phosphatase (ALP), Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined in the serum using standard assay kits from Randox (for creatinine, Urea, and ALT) and Teco (for ALP and AST) chemicals.

Statistical Analysis: The SPSS 20.0 software was employed for data entry and validation. Statistical analysis was carried out between the treatment group and control using the student t-test. A $P \leq 0.05$ was considered statistically significant.

RESULTS

HISTOLOGY OF THE KIDNEY SECTIONS

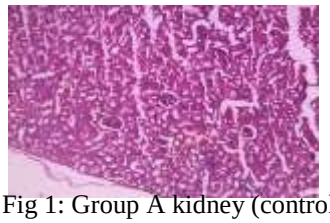

Fig 1: Group A kidney (control) H&E X100
Section shows normal proximal tubules, glumeruli, interstitial spaces and blood vessels. The tubules are lined by regular cubiodal epithelium. The glomeruli, blood vessels and interstitium are normal.

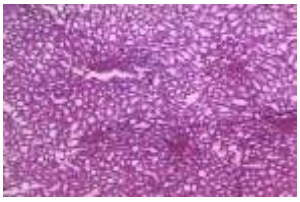

Fig 2: Group B kidney 300mg/kg H&E X100.
Section shows mild interstitial filtration by inflammatory cells and mild vascular congestion.

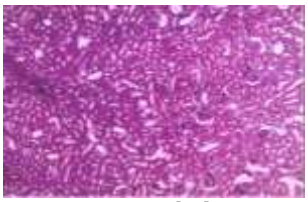

Fig .3: Group C kidney 400mg/kg H&E X100
Section shows mild inflammatory reaction and mild vascular congestion, the interstitial space does not appear remarkably.

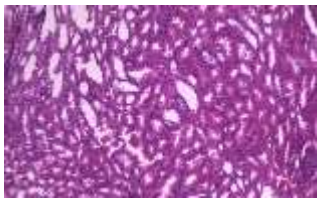

Fig. 4: Group D kidney 500mg/kg H&E x100
Section shows mild to severe inflammatory reaction and increased vascular congestion. The interstitial spaces are not remarkably

HISTOLOGY OF THE LIVER SECTIONS

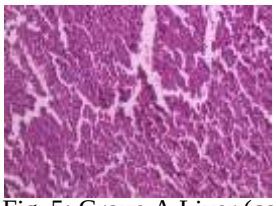

Fig .5: Group A Liver (control) H&E X100
Section shows a normal liver lobule in which there are plates of hepatocytes separated by sinosoids, also seen are the portal triad and central vein.

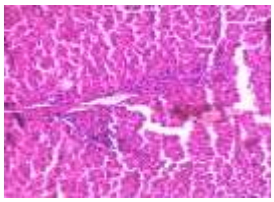

Fig 6: Group B Liver 300mg/kg H&E X100.
Hepatocytes and portal triads with mild periportal inflammatory cell infiltrates.

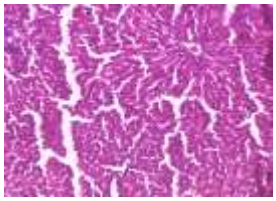

Fig 7: Group C Liver 400mg/kg H&E X100
Section of the liver shows mild periportal inflammatory cell infiltrates, it also show chronic inflammatory cells.

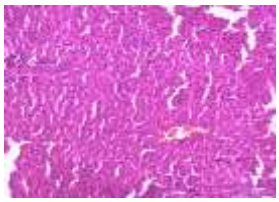

Fig. 8: Group D Liver 500 mg/kg H&E x100
Section of the liver shows mild to severe periportal inflammatory cell infiltrates; it also shows chronic inflammatory cells.

Table 1: Effect of aqueous extract of Ocimum gratissimum on the body weight of Wistar rats

The pattern of weight changes in the various groups of the Wistar rats before the treatment and during the treatment are shown in this table.

Groups	Before treatment	Day 7	Day 14	Day 21
Group A (control)	137.50±6.29	140.00±8.42	151.25±9.44	147.50±10.90
Group B	161.25±3.75	158.75±4.27	162.00±3.14	160.50±4.77
Group C	157.50±4.79	161.25±4.27	162.50±5.20	166.25±5.15
Group D	172.50±1.44	176.25±3.15	183.25±3.61	191.25±3.15

Values are expressed as mean ± standard error of mean (S.E.M), n=6.

Table 2: Effect of aqueous extract of Ocimum gratissimum on serum Creatinine, Urea, AST, ALP, and ALT levels in Wistar rats

Average serum levels of creatinine, urea, aspartate aminotransferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP) treated rats relative to the control group.

Groups	Creatinine (mmol/I)	Urea (mmol/I)	ALP (u/I)	AST (u/I)	ALT (u/I)
Group A control	173.97±0.43	16.10±0.62	44.93±0.12	30.00±10.58	7.33+0.58
Group B	175.00±0.55	16.23±0.24	46.80±2.16	32.33±0.67	7.67+0.88
Group C	175.57±0.29	16.47±0.26	44.10±1.64	29.00±0.58	6.33+0.67
Group D	175.57±1.85	16.50±0.12	42.20±0.38	27.33±0.88	1.33+0.33

Values are expressed as mean + standard error of mean (S.E.M), n=6.

Creatinine, urea, Alkaline phosphatase (ALP), Aspartate Amino Transferase (AST), Alamine Amino Transfarase (ALT), levels in rats used to determine effects of aqueous extract of Ocimum gratissimum in the kidney and liver expressed in Mean ± SEM., From table 2, Creatinine level of Control group showed no significant increase ($P>0.05$) when compared with test Groups B, C and D.

DISCUSSION

In many parts of the world, especially Africa and Asia, plant parts are used for the treatment of various ailments such as inflammation, fever, gout (krawinkel). The leaves of Ocimum gratissimum is used for prevention and treatment of gout, catarrh, fever and malaria which has been found to be associated with free generation of radicals (Pamplona, 2004). Phytochemical evaluation of this plant has shown that it is rich in alkaloid, saponin, tannin, phytates, flavonoids and oligosaccharides and contains tolerable gyanogenic substances (Ijeh, et al., 2004). The medicinal values of the Ocimum gratissimum lie in their component phytochemical which produces physiological actions on human body (Afolabi, et al., 2007). The result of this study shows that there was no significant difference in the weights of the Wistar rats in the treatment Groups B, C, and D with the control group over the 3 weeks period of administration of the extract and this result was in agreement with the works of Eboh and Ekundina (2012). Ocimum gratissimum reduced the level of serum ALT, AST and ALP activities in the liver compared to rats in the control group. The effect was dose dependent and this agrees with Effraim et al., (2000). Flavonoids are reported to exhibit antioxidant activity (Ramanathan et al., 1989) and hepato-protective (Seevola, et al., 1984). The elevation of kidney enzymes, urea and creatinine levels in serum is taken as the index for nephrotoxicity (Patwardhan et al., 2005). From the study Ocimum gratissimum maintained the level of serum urea and creatinine. This is as a result of a probable nephroprotective effect of Ocimum gratissimum).. The kidney of treated rats revealed vascular congestion, and unremarkedable appearance of the interstitial spaces and glomeruli with varying degrees of interstitial infiltration by inflammatory cells, which correspond with the study of (Adejoke et al., 2012).

Saponin is known to be toxic to the body system and causes vascular congestion (Brandurijk et al., 1962). Glomerular filtration may be reduced as a result of the impairment of renal function which can be caused by any acute condition. This study concludes that while Ocimum gratissimum may be useful in culinary dishes and also used in the treatment of certain ailments, systemic toxicity is also possible and this is dose dependent. Caution should therefore be exercised in the therapeutic use of the plant.

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