Methanol extract of Ocimum gratissimum leaves modulates the liver and kidney functions in CCl_4 – induced hepatotoxicity in albino rats.

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Abstract

Ocimum gratissimum is used traditional plant in several disorders due to their excellent antioxidant properties. In a 14 - day study, animals were divided into six groups (A-F) of five rats each. Group A (normal control), group B was given CCl_4 only while groups C, D, E and F were given CCl_4 with various doses of methanol extract of *Ocimum gratissimum* leaves (100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg) body weight respectively. Effect of daily intake of methanol extract of *Ocimum gratissimum* leaves over a period of two weeks on rats' hepatocellular system was investigated. Standard enzyme assays were conducted for some enzymes of the liver and kidney. From the result, there was relatively no significant difference ($p<0.05$) in enzyme activity of the groups given the extract and group A in both liver and kidney. It could be inferred therefore, that methanol extract of *Ocimum gratissimum* leaves could serve as excellent chemotherapeutic agent for prevention of CCl_4 – induced hepatocellular damage.

Keywords: Ocimum gratissimum leaves, carbon tetrachloride, methanol extract, hepatotoxicity, enzymes.

INTRODUCTION

Ocimum gratissimum, among other indigenous plants has being of immense contribution to the health care in Nigeria[1,2]. It is used for medicinal, condiment and culinary purpose. Its flowers and the leaves are rich in essential oils, thus, it is used in preparation of teas and infusion [3]. Phytochemical screening showed that methanol extract is rich in tannins, steroids, terpenoids, flavonoids and cardiac glycosides which make it an excellent free radical scavenging agent[4]. Phytochemicals are effective in preventing or combating diseases due to their antioxidant effects[5]. Antioxidants protect other molecules from free radicals attack which have been implicated in the pathogenesis of several diseases[6].

CCl_4 is a classical hepatotoxin that causes rapid liver damage progressing from steatosis to centrilobular necrosis. The mechanism of liver damage induced by CCl_4 is thought to involve free radicals generation and lipid peroxidation[7].

The present research directed efforts on evaluating the action of methanol extract of *Ocimum gratissimum* leaves on the CCl_4-induced hepatotoxicity in male albino rats.

MATERIAL AND METHODS

Animals

Thirty male albino rats weighing between 150±200g used for this study were obtained from animal holding of Salem University, Lokoja, Nigeria. The rats were housed in standard cages under standard environmental conditions (temperature, relative humidity and light/day cycle). They were divided into six groups comprising five animals each. Feed and water were given the rats *ad libitum*.

Preparation of Methanol Extract of the Plant

The leaves of *Ocimum gratissimum* were collected, air dried and ground in Ibadan, Oyo State. 750g dried weight of the leaves was soaked in methanol (5L: 500g) for 48hrs and filtered with muslin cloth. It was concentrated in the Central Research Laboratory of University of lbadan, Oyo state, using rotary evaporator.

Experimental design

1.5ml/kg body weight of carbon tetrachloride, CCl_4, was used to induce hepatotoxicity (p.o) in the rats (1:1, v/v CCl_4/Olive oil)[8]. The rats were divided into six groups of five animals each. Group A: rats given only the extract vehicle (Distil water), group B: received only carbon tetrachloride, groups C, D, E and F received

carbon tetrachloride (CCl_4) and methanol extract of *Ocimum gratissimum* leaves at doses of 100mg/kg , 200mg/kg, 300mg/kg and 400mg/kg body weight respectively. The extract was administered orally for the period of fourteen days.

Blood Collection

The rats were fasted overnight, anaesthetized in a jar containing chloroform and sacrificed by jugular puncture after fourteenth day. The blood was collected in an anticoagulant free bottle and centrifuged at 3500rpm for 15 minutes using refrigerated centrifuge RC650s and the serum obtained was preserved at -8^{o}C until required for use.

Biochemical Analysis

Estimation of Liver function markers

Gamma-glutamyltransferase (GGT) quantitative determinations were done spectrophotometrically using Tietz method[9] with Randox laboratory test Kit. The estimation of Aspartate aminotransferase (AST) was done by Reitman and Frankel method[10] for the quantitative estimation of serum using Randox laboratory test Kit (Antrim, UK). Alkaline phosphatase (ALP) determination was carried out using Englehardt method[11] for the quantitative measurement of serum using Randox laboratory test Kit (Antrim, UK).

Estimation of kidney function markers

Creatinine level was determined using Schirmeister, *et al.*, method[12] with Randox laboratory test kit (Antrim, UK). Chaney and Marbarch method[13] was used to investigate the urea concentration spectrophotometrically using Randox laboratory test kit (Antrim, UK).

Data Analysis

The results were expressed as mean ± S.D of five animals from each group. The data were evaluated by one way ANOVA using SPSS version 20. P value < 0.05 was considered statistically significant**.**

RESULTS

Figure 1 displays the activity of gamma-glutamyltransferase in CCl_4- induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves. There was significant increase ($p<0.05$) in the activity of GGT in serum of the group B (CCl_4 only) relative to the control group A. Whereas no significant difference ($p<0.05$) in the activity of GGT in serum of group C (CCl_4 +100mg/kg body weight methanol extract of *O.g* leaves), D (CCl_4 +200mg/kg body weight methanol extract of *O.g* leaves), E (CCl_4 + 300mg/kg body weight methanol extract of *O.g* leaves) and F (CCl_4 + 400mg/kg body weight methanol extract of *O.g* leaves) relative to the control group A was observed.

Figure 2 illustrates the activity of Aspartate Aminotransferase (AST) in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves. There was significant difference ($p<0.05$) in the activity of AST in serum of group B and C relative to the control group A. Conversely, no significant difference ($p<0.05$) in serum AST activity of group D, E and F compared to the control group was evident.

Figure 3 shows the urea concentration in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves. There was significant rise ($p<0.05$) in the level of urea in serum of groups B and C relative to the control group A. In contrast, there was no significant difference ($p<0.05$) in the serum urea of group D, E and F relative to the control group A.

Figure 4 displays the creatinine level in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves. There was significant increase ($p<0.05$) in the serum creatinine concentration in group B relative to the control group A. Conversely, no significant difference ($p<0.05$) in the serum creatinine concentration in group C, D, E and F relative to the control group A was observed.

Figure 5 presents the activity of alkaline phosphatase (ALP) in CCl_4 –induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves. There was significant increase ($p<0.05$) in the serum alkaline phosphatase activity of group B relative to the control group A while there was no significant difference ($p<0.05$) in the activity of alkaline phosphatase in serum of group C, D, E and F relative to the control group A.

DISCUSSION

CCl_4 is a common hepatoxin used for the screening of hepatoprotective therapy. The CCl_4 is converted into reactive metabolite, halogenated free radicals by the hepatic cytochrome P_{450} system which in turn covalently binds to cell membrane and organelles to cause serious damage to the system[14,15].

An enormous body of research supports the recommendation that antioxidant therapy is an attractive approach for amelioration of diverse ailments such as liver damage, kidney damage, cardiovascular diseases and diabetes. The liver is necessary for survival and plays a major role in metabolism as well as in numerous biochemical processes like decomposition of erythrocytes, glycogen storage, plasma protein synthesis and detoxifications[16].

The elevated serum GGT, AST and ALP activities of group B rats observed when compared with the control group may result from the interference of the CCl_4 with the structural integrity of the liver[17,18]. It is also an indication that CCl_4 could have induced a progressive damage at the centrilobular junction in the hepatocytes of the rats[19,20]. The decrease observed in GGT, AST and ALP in the treated rats may result from hepatoprotective potency of *Ocimum gratissimum* on the liver cells following restorations of its cell membrane permeability[21]. This ameliorative effect is indicative of the decline in the activities of the enzyme markers in the extracellular milieu of the hepatocytes. This is in support of the findings of Ujowundu, *et al.*[22], that *O. gratissimum* has chemopreventive potential against diesel induced hepatotoxicity in rats.

The increase serum urea and creatinine concentrations of group B rats observed may result from the interaction of the CCl_4 with the kidney cells which reduced the creatinine and urea clearing capacity of the kidney. This may stem from the loss of structural integrity of the kidney cell membrane[23,24]. Also the elevated concentration of urease in the treated group C may be an indication of the saturation propensity of CCl_4 which prevented the protective efficacy of the low dose of the extract. Administration of methanol extract of *Ocimum gratissimum* leaves reduced the level of urea in group D, E and F in dose-dependent manner.

Drastic reduction of creatinine level observed in all the treatment groups would imply a possible repair in the kidney cell impairment caused by CCl_4.

This study showed that CCl_4 intoxication could lead to hepatocellular and nephrocellular damage and also generate reactive oxygen species (ROS). It can also be deduced that phytochemical and antioxidant components of *O. gratissimum* can ameliorate and revamp structural and functional integrity of the liver and kidney.

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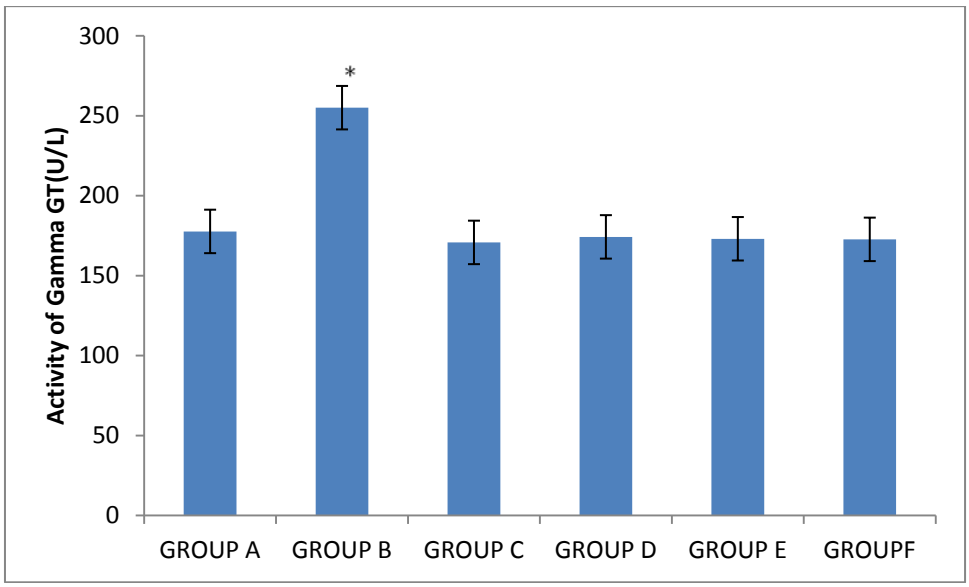

Figure 1: Activity of GGT in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves over a period of two weeks. Plotted results are means of five determinations ± SD. Bar with asterisk shows a significant different ($p<0.05$) from the control group A.

Group A: Rats given only the extract vehicle (Distil water), Group B: Rats were treated with CCl_4 only, Group C: Rats treated with CCl_4 +100mg/kg body weight of extract, Group D: Rats treated with CCl_4 + 200mg/kg body weight of extract, Group E: Rats treated with CCl_4+ 300mg/kg body weight of extract, Group F: Rats treated with CCl_4+ 400mg/kg body weight of extract.

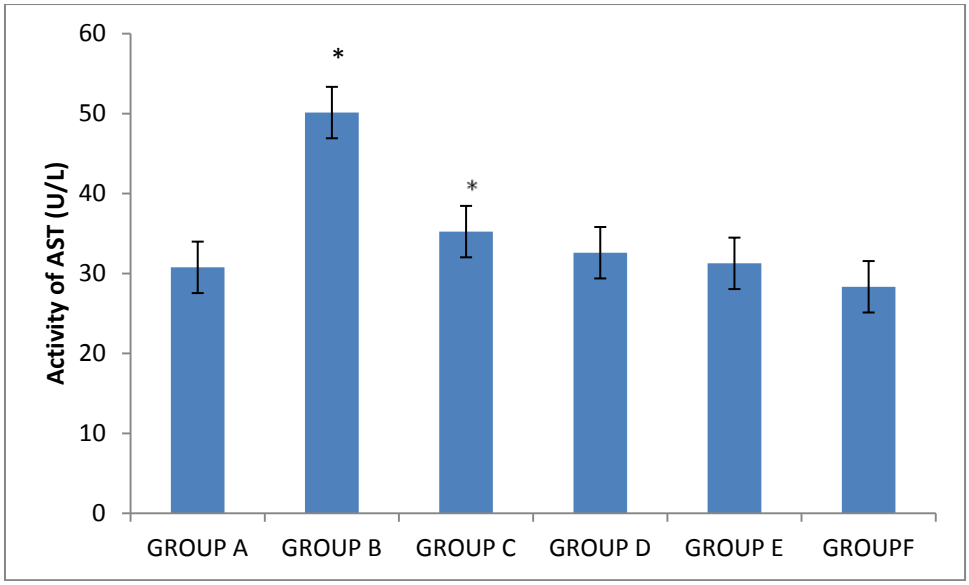

Figure 2: Activity of AST in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves over a period of two weeks. Plotted result are mean of five determinations ± SD. Bars with asterisk shows a significant difference ($p<0.05$) from the control group A.

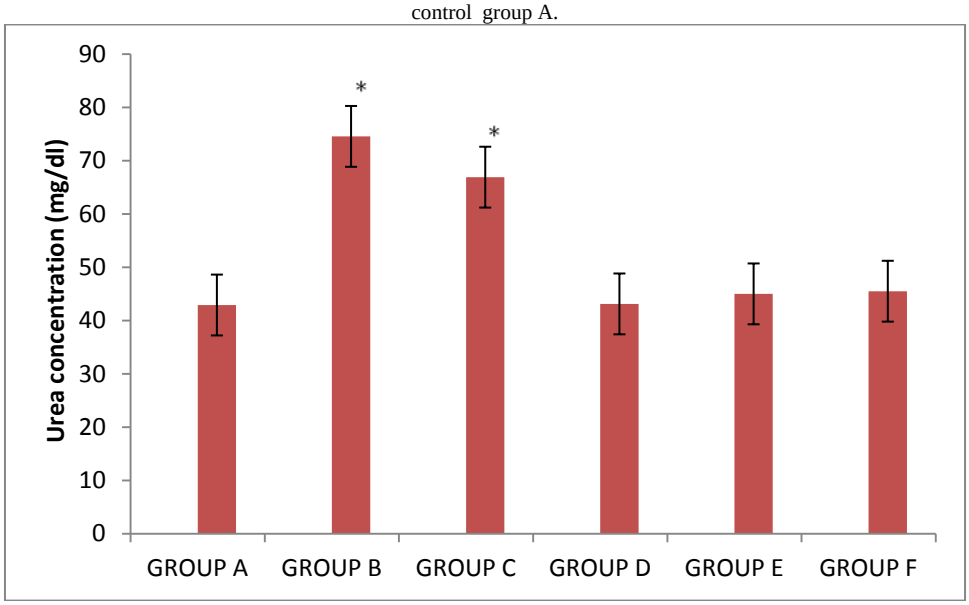

Figure 3: Urea concentration in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves over a period of two weeks. Plotted results are means of five determinations ± SD. Bars with asterisk shows a significant different ($p<0.05$) from the control group A.

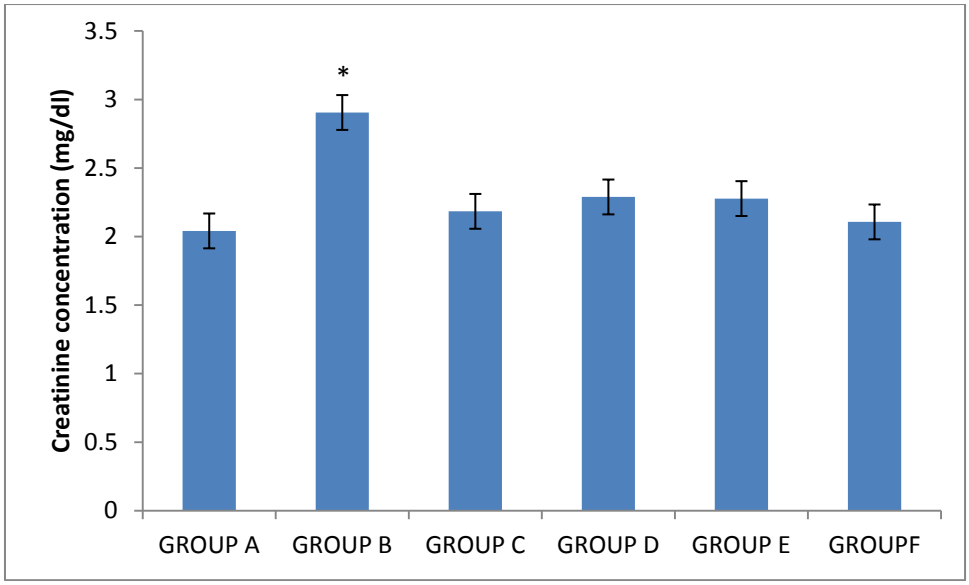

Figure 4 Creatinine concentration in CCl_4 – induced hepatotoxicity in rats placed on methanol extract of *Ocimum gratissimum* leaves over a period of two weeks. Plotted result are means of five determinations ± SD. Bar with asterisk shows a significant different ($p<0.05$) from the control group A.

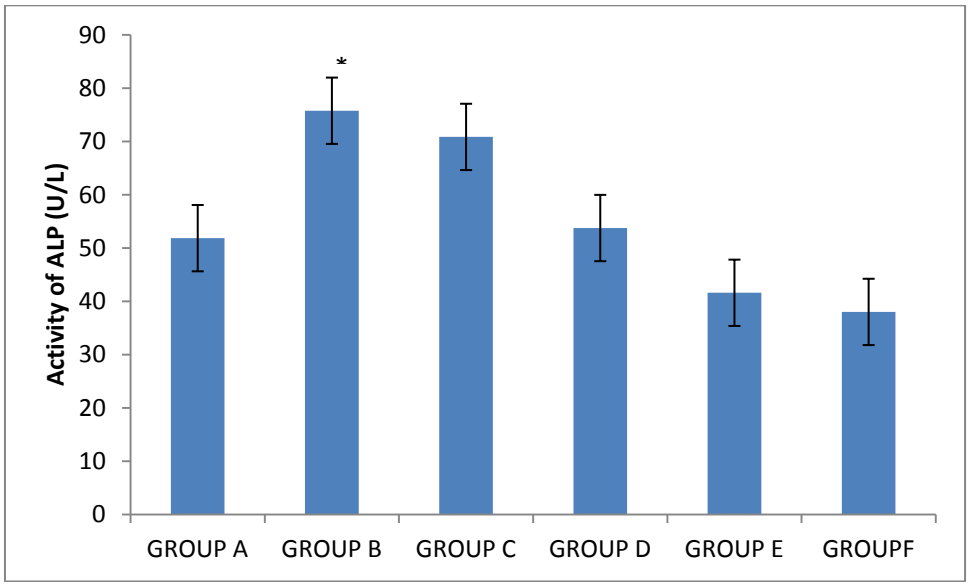

Figure 5 Activity of ALP in CCl_4 – induced hepatotoxicity in rats placed on methanol of extract *Ocimum gratissimum* leaves over a period of two weeks. Plotted results are means of five determinations ± SD. Bar with asterisk shows a significant difference ($p<0.05$) from the control group A.

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