Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20160320033421

Original Research

# Potential hepatoprotective effect of different solvent fractions of *Ocimum gratissimum* (O.G) in a paracetamol - induced hepatotoxicity in Wistar albino rats

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**Received:** January 14, 2016
**Accepted:** February 24, 2016
**Published:** March 29, 2016

## ABSTRACT

**Objective:** To conduct a potential hepatoprotective effect of different solvent fractions of *Ocimum gratissimum* (OG) On paracetamol-induced hepatotoxicity in Wistar albino rats. **Methods:** The animals were divided into seven groups of five animals per group. Groups I and II served as normal and negative controls respectively. Group III received 100mg/kg b.w silymarin (SIL) and served as positive control. Group IV received 400mg/kg b.w ethanol fraction; Group V received 400mg/kg b.w ethyl acetate fraction; GroupVI received 400mg/kg b.w hexane fraction; Group VII received 400mg/kg b.w aqueous fraction. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alanine phosphatase(ALP),Total Protein, Total and direct bilirubin and histopathological examination of the liver were conducted using a standard biochemical methods. **Results:** Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alanine phosphatase(ALP) activities were significantly ($p \leq 0.05$) decreased in groups treated with different fractions of ocimum gratissimum when compared with the negative control. Total bilirubin and direct bilirubin concentrations decreased significantly ($p \leq 0.05$) in groups treated with different fractions of OG when compared with the negative control. Total protein concentration increased significantly ($p \leq 0.05$) in hexane and aqueous fractions compared with negative control. Histopathological examination of the liver sections corroborated the data from biochemical studies. **Conclusion:** These findings showed that different fractions of Ocimum gratissimum extract may exhibit hepatoprotective properties.

KEY WORDS:Ocimum gratissimum; Paracetamol; Hepatotoxicity; Liver.

## INTRODUCTION

Herbal medicines tend to look primitive and unscientific when compared to synthetic (conventional) drugs which are thought to be more reliable than those made from plants. Herbal medicines is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care [1]

*Ocimim gratissimum* (OG) belongs to the family Lammiaceae. It is commonly called alfavaca and is cultivated in many gardens around village huts in Nigeria for its medical and culinary uses [2]. Phytochemical screening of this plant has revealed the presence of many active ingredients, such as flavonoids, triterpenes, alkaloids, saponins, eugenol, linaol, methyl cinnamate, camphor and thymol [3][4]. Eugenol, an isolate from OG has been observed to posses antihelminthic, nematicidal and insecticidal properties [5] [6].

Several species and varieties of the genus *ocimum* have been reported to yield essential oils, the oils are active against several bacteria (including staphylococcus avreus, listeria monocytogenes, Escherichia coli, etc) and fungi (including trichophyton rubum, T. mentagrophytes etc. [7] [8] [9] [10] [11]

It showed various medicinal potentials in chemopreventive, anticarcinogenic, free radical scavenging and reduces protective uses [12] [13] [14]. Additionally, ethanol extract of *ocimum gratissimum* leaf also revealed significantly chemopreventive effects on chemical- induced papilomogenesis by modulating metabolizing enzymes such as glutathione–s-transferase and aryl hydrocarbon hydroxylase [15][16]. The liver is the largest organ in human body and necessary for metabolism of drugs and exogenous toxins. Liver damage is a prevalent pathology that involves a variety of disorders including oxidative stress, steatosis, hepatitis, fibrosis cirrhosis, apoptosis and hepatocellular carcinoma [17].However, liver damage due to natural industrial toxins or drugs is common but rarely recognized [18]. Various xenobiotics are known to cause hepatotoxicity such as carbon tetrachloride ($CCl_4$) and paracetamol [19], which alters the antioxidant profile of the liver including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione transferase (GSH) [20]. It is also responsible for a leading cause of death in so many cases of transplant [21].

## MATERIALS AND METHODS

### Collection and Identification of Materials

*Ocimum gratissimum* leaves were purchased from Ubani Market in Umuahia, Abia State. They were identified by the Herbaruim unit of the department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. Umuahia , Abia State.

### Preparation of Fractions of Extract

All the leaves were washed in running tap water, air dried and pulverized. The powdered material each was directly macerated in different solvents; n –hexane, ethylacetate, ethanol and water for 48 hours with intermittent shaking to facilitate extraction. The different extracts were filtered using whatman filter paper and were evaporated to dryness using rotary evaporator. The residues obtained were dissolved in olive oil.

A stock solution of 0.1g/ml of each extract was prepared and stored in the refrigerator. The volume of stock to be administered based body weights was calculated according to the formula.

$$VOL = \frac{D \times P}{C}$$

D = dose to be administered, P = body weight of the animal in Kg, concentration of the stock.

## EXPERIMENTAL PROCEDURES

### Experimental design

Wistar albino rats of both male and female sex weighing between (60-120g) were used for this study. The animals were obtained from the Animal Breeding Unit of the College of Veterinary medicine, University of Nigeria, Nsukka. They were kept in well ventilated plastic stainless steel cages and left under laboratory conditions for two (2) weeks for acclimatization. The animals were divided into seven groups (i-vii) of five rats each. They were fed with commercial rat feed and clean tap water *ad libitum* throughout the period of experiment. Group 1 was the control, administered only olive oil, group II was treated with Paracetamol only, group III was treated with standard drug (silymarin) and paracetamol, group IV; ethanol extract and paracetamol, group V; ethylacetate extract and paracetamol, group VI; hexane extract and paracetamol and group VII; water extract and paracetamol. At the end of the treatment period, rats in all the groups were dissected and blood samples were collected by cardiac puncture.

### Acute toxicity study

The acute toxicity test was carried out using Ten (10) albino rats weighing between (60-120) g. The rats were randomly distributed into (5) groups; each group had two (2) rats. They were fasted overnight and weighed before the extracts were administered. According to the Organization for Economic and Cooperative Guidelines 425 (OECD guidelines, 2000), relative doses of 100mg/kg body, 250mg/kg bodyweight , 350 mg/kg bodyweight 500mg/kg body weight, 1000kg/kg bodyweight,1500mg/kg bodyweight and 2000mg/kg body weight of the extracts were given within 24 hours to the rats by oral galvage for seven days. Group 1 (Control); Group II (aqueous extract); Group III (Ethanol extract), Group IV (Ethyl acetate extract), Group V (n-Hexane extract). The rats were observed for few hours on fasting after dosing to record any death, changes in appearances, discoloration of furs, skin, eyes, mucous respiratory and other physiological signs [22] [23] [24]

### Preparation of drugs

Stock solution of paracetamol, 500mg/kg body weight [22] and silymarin 100mg/kg body weight [25] were prepared respectively and administered to the animals based on their body weights.

### Induction of hepatotoxicity

Hepatotoxicity was induced by administering 500mg/kg body weight of paracetamol everyday for 28 days.

**Sub-chronic study**

**Group I:** (Control) was treated with 1ml of olive oil only.

**Group II:** (Paracetamol alone) was treated with 500mg/kg body weight of paracetamol (PCM) dissolved in 1ml of olive oil

**Group III**: was treated with100mg/kg body weight of silymarin in (SIL) + 500mglkg body weight of paracetamol (PCM + SILYMARIN) dissolved in 1ml of olive oil.

**Group IV**: was treated with 400mg/kg body weight of ethanol fraction of *ocimum gratissimum* + 500mg/kg body weight of paracetamol in dissolved in 1ml of olive oil (PCM + ETHANOL)

**Group V:** was treated with 400mg/kg body weight of ethylacetate fraction of *ocimum gratissimum* + 500mg/kg body weight of paracetamol dissolved in 1ml of olive oil (PCM + ETHYLACETATE)

**Group VI:** was treated with 400mg/kg body weight of n-hexane fraction of *ocimum gratissimum* + 500mg/kg body weight of paracetamol dissolved in 1ml of olive oil (PCM + N-HEXANE)

**Group VII:** was treated with 400mg/kg body weight of aqueous fraction of *ocimum gratissimum* + 500mglkg body weight of paracetamol dissolved in 1ml of olive oil (PCM + AQUEOUS)

### Collection of blood samples.

Blood samples were collected in EDTA free bottles for Biochemical assay.

### Chemicals

All the chemicals used in this study were of analytical grade.

## BIOCHEMICAL ANALYSIS

### Aspartate Aminotransferase (AST) Determination

The activities of AST were determined using [26] method.

### Alanine Aminotransferase (ALT) Determination

The activities of ALT were determined using [26] method

### Alkaline Phosphatase (ALP) Determination

The activities of ALT determined using standard colorimetric methods of [27].

### Serum Bilirubin Determination

The method used for the determination of serum bilirubin was as described by [28].

### Total Protein Determination

It was carried out as described by [29]

### Histological examination

The liver was excised, washed in 0.9% saline, weighed and transferred into ice cold containers for biochemical assay. The excised liver tissues were collected in a sterile universal container containing 10% neutral formalin. They were processed and embedded in paraffin wax to provide a hard support for sectioning. Every third section was mounted in glass slide and stained with hematoxylin and eosin and photomicrographed

### Statistical Analysis

Descriptive statistics were carried out on the data generated. Result were expressed as the Mean ± S.E.M. one way analysis of variance (ANOVA) was used to separate means with post-hoc multiple comparism (option - LSD). Probability values is less than or equal to 0.05 ($p \leq 0.05$). Data analysis was done using SPSS (statistical package for social scientists).

## RESULTS

### Result of Acute Toxicity

There was no death recorded at the dose of 2000mg/kg body weight but the rats showed slowness in activity next day. Thus, one fifth of this dose (400mg/kg body weight) was used as the highest dose for this study.

**Effect of different fractions of *ocimum gratissimum* on serum liver marker enzymes activities in Wistar albino rats as shown Table 1.**

The result of effect of different fractions of *Ocimum gratissimum* on serum enzyme liver marker enzymes activities of Wistar rats showed that there was a significant ($p \leq 0.05$) increase in Serum liver marker enzymes (AST, ALT, ALT) activities in group treated with PCM alone (Group II) when compared with control group (group I). However groups administered with different fractions of OG (Groups IV-VII) and silymarin (Group III) showed a significant decrease ($p \leq 0.05$) in Serum liver enzymes activities when compared with group treated with PCM alone.

**Table: Effects of different fractions of *Ocimum gratissimum* on Total Protein, Total and Direct Bilirubin as shown in Table 2**

The result of effects of different fractions of *Ocimum gratissimum* on Total Protein, Total and Direct Bilirubin as shown in Table 4.1.3 showed that there was a significant ($p \leq 0.05$) decrease in Total Protein concentration in group treated with PCM alone (GP II) when compared with control group. However, there was a significant ($p \leq 0.05$) increase in groups treated with hexane and aqueous fractions of O.G when compared with group treated with PCM alone while Silymarin and ethanol fraction of OG (ETH ) showed a marked increase when compared with group treated with PCM alone (GP II) .On the other hand, total and direct bilirubin concentration showed a significant ($p \leq 0.05$) increase in group treated with PCM alone (GP II) when compared with control group( GP I), However groups treated with different fractions of OG and Silymarin group showed a significant ($p \leq 0.05$) decrease when compared with group treated with PCM alone.

**Table 1.** Effect of different fractions of *Ocimum gratissimum* on serum liver marker enzymes activities in Wistar albino rats.

| GROUPS | TREATMENT | AST(IU/L) | ALT(IU/L) | ALP(IU/L) |
|---|---|---|---|---|
| I | CONTROL | $132.60 \pm 4.41^{ad}$ | $68.80 \pm 1.62^{ad}$ | $15.00 \pm 0.54^{ad}$ |
| II | PCM | $208.20 \pm 6.85^{bc}$ | $119.20 \pm 4.04^{bc}$ | $22.00 \pm 1.04^{bc}$ |
| III | PCM+SIL | $130.00 \pm 5.87^{ad}$ | $106.80 \pm 1.85^{bd}$ | $19.00 \pm 0.31^{bd}$ |
| IV | PCM+ETH | $134.20 \pm 1.93^{ad}$ | $106.20 \pm 1.15^{bd}$ | $19.20 \pm 0.73^{bd}$ |
| V | PCM+ACE | $124.60 \pm 4.06^{ad}$ | $84.40 \pm 4.84^{bd}$ | $16.40 \pm 1.03^{ad}$ |
| VI | PCM+HEX | $126.20 \pm 6.61^{ad}$ | $81.00 \pm 2.30^{bd}$ | $17.60 \pm 1.24^{bd}$ |
| VII | PCM+AQU | $156.60 \pm 3.94^{ad}$ | $101.20 \pm 2.57^{bd}$ | $15.80 \pm 0.86^{ad}$ |

Values are expressed as means ±S.E.M (n=5). Values with different superscripts letter (a, b) in the same row are significantly different ($p \leq 0.05$) when comparing group I with other groups.
Values with different superscripts letter (c, d) in the same row are significantly different ($p \leq 0.05$) when comparing group II with other groups.

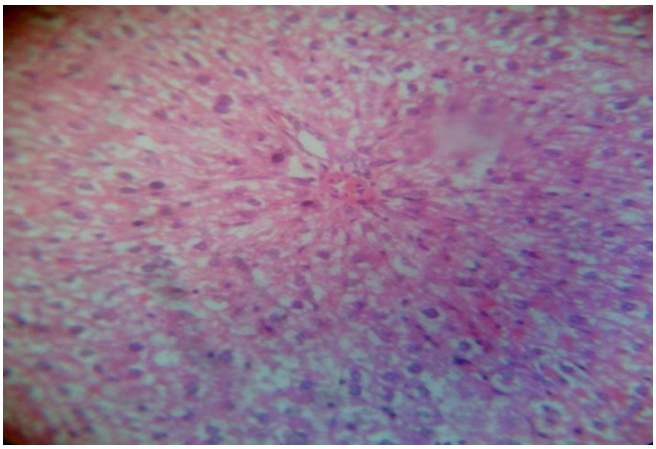
**Table 2.** Effect of different fractions of *Ocimum gratissimum* on Total protein, Total and Direct bilirubin in Wistar albino rats.

| GROUP | TREATMENT | TOTAL PROTEIN(mg/dl) | TOTAL BILIRUBIN(mg/dl) | DIRECT BILIRUBIN(mg/dl) |
|---|---|---|---|---|
| I | CONTROL | 5.76±0.19$^{ad}$ | 0.728±0.062$^{ad}$ | 0.136±0.038$^{ad}$ |
| II | PCM | 4.72±0.20$^{bc}$ | 1.544±0.080$^{bc}$ | 0.270±0.045$^{bc}$ |
| III | PCM+SIL | 5.38±0.17$^{ac}$ | 0.922±0.043$^{bd}$ | 0.194±0.042$^{bd}$ |
| IV | PCM+ETH | 4.92±0.28$^{bc}$ | 0.836±0.033$^{ad}$ | 0.160±0.031$^{ad}$ |
| V | PCM+ACE | 4.42±0.16$^{bc}$ | 0.818±0.063$^{ad}$ | 0.142±0.014$^{ad}$ |
| VI | PCM+HEX | 5.90±0.23$^{ad}$ | 0.408±0.029$^{bd}$ | 0.110±0.024$^{ad}$ |
| VII | PCM+AQU | 6.50±0.38$^{bd}$ | 0.664±0.033$^{ad}$ | 0.192±0.021$^{bd}$ |

Values are expressed as means ±S.E.M (n=5). Values with different superscripts letter (a, b) in the same row are significantly different (p≤0.05) when comparing group I with other groups.
Values with different superscripts letter (c, d) in the same row are significantly different (p≤0.05) when comparing group II with other groups.

## Histopathological Examination Results

Histopathological examination of the liver tissues showed that the normal control group (Group I) had normal liver architecture compared with negative control group (PCM only) that showed severe sinusoid enlargement, congestion, mononuclear infiltration and hemorrhage. Administration of different fractions of *ocimum gratissimum* extract and Silymarin minimizecongestion, mononuclear infiltration and cytoplasmic vacuolation of the hepatocytes caused by paracetamol intoxication.



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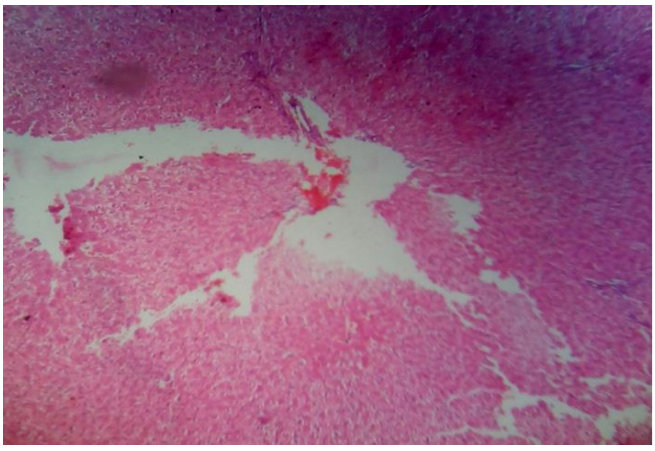

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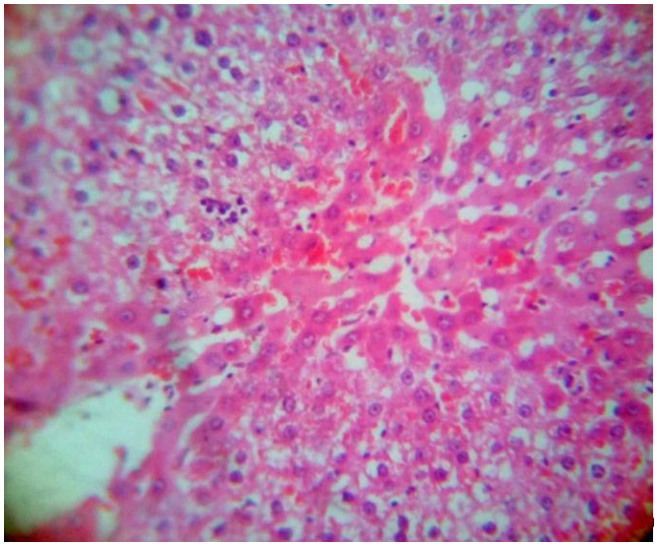
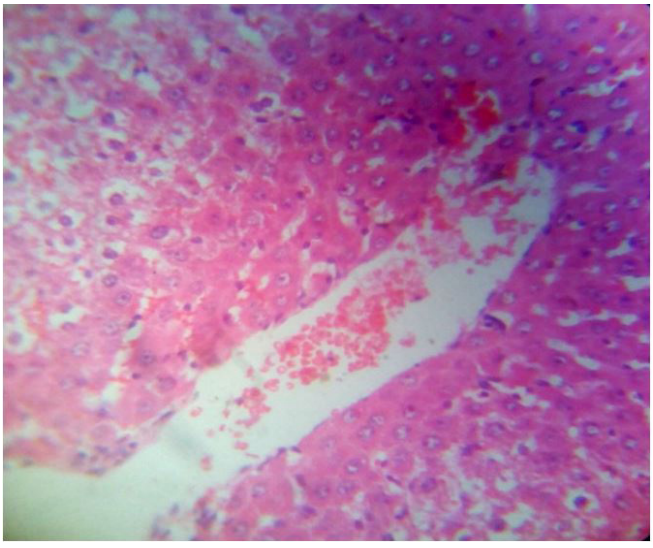
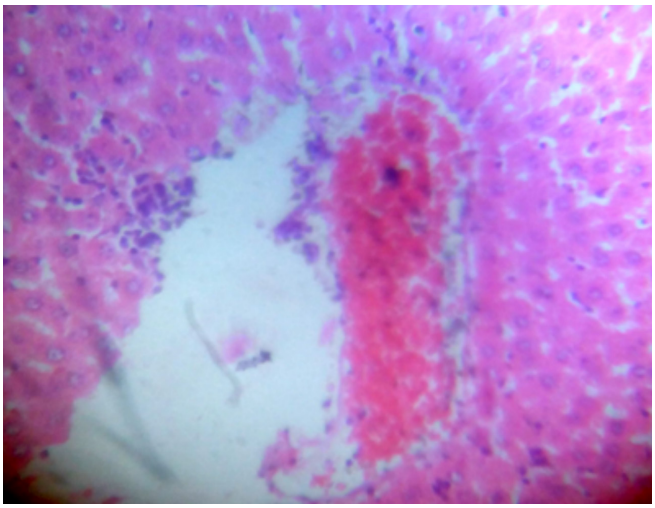


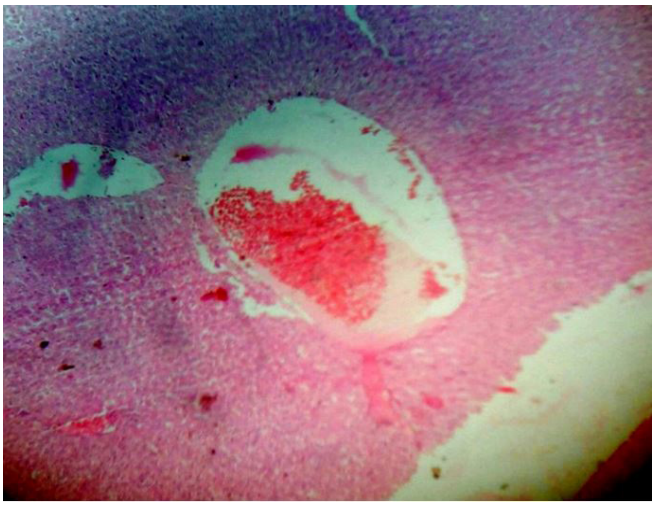
**PCM+ SIL**



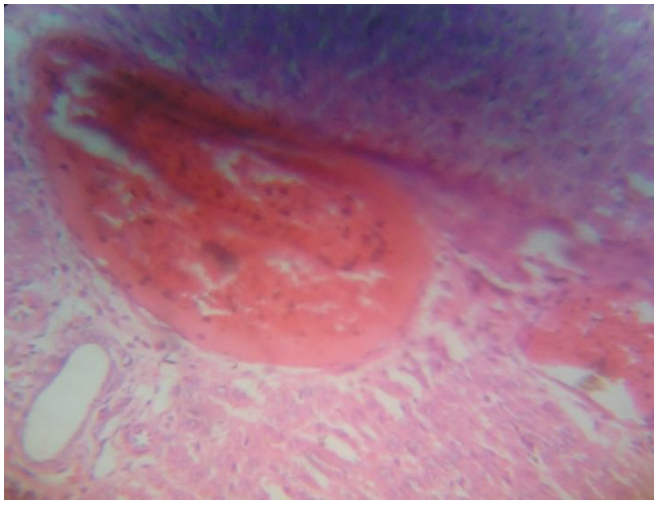
**PCM+ETH**



PCM+ACE



PCM+HEX



PCM +AQU

## DISCUSSION

The result of the acute toxicity showed that no sign of toxicity and mortality was recorded at the administration of different fractions of *ocimum gratissimum* extracts. This however implies that $LD_{50}$ may be greater than 2000mg/kg bodyweight. The extract used may be considered to be slightly or practically non toxic according to dose response classification. One fifth of 2000mg/kg body weight (400mg/kg) of the extract from the different fractions of *ocimum gratissimum* was therefore used for this study as a fixed dose.

Alteration in the biochemical indices of organ function impairs the normal functioning of the organs[30].The assessment of biochemical parameters such as liver and kidney function in the serum of animals following the administration of chemical, including plants, plays a significant role in the evaluation of the toxicity risk or safety of such compound [31] [32]and the liver being the primary organ for detoxification and distribution could be assessed to establish the safety or toxicity of a substance(could be drug or plant) [33].

Serum enzyme measurements are valuable tools in clinical diagnosis and provide information on the effect and nature of pathological damage to any tissue. Biomolecules which did not originate from the serum migrate into the serum through leakage. Alanine and aspartate aminotransferase (ALT and ALP) are cytosolic in origin [34] while alkaline phosphatase (ALP) is an enzyme of the plasma membrane). Aspartate aminotransferase is another enzyme associated with liver parenchyma cells, its activity is located in the microsomal and mitochondrial portion of the liver cells as well as in the skin, skeletal and cardiac muscles, pancreas and kidney. The rate of AST to ALT is sometimes useful in differentiating between the causes of liver damage [35]. Alanine transaminases (ALT) is an enzyme present in hepatocytes (liver cells), it leaks out into the blood where it is measured. It has its highest concentration in the liver with kidney and skeletal muscle having less activity of the enzyme. Its activity rises dramatically in acute liver damage such as viral hepatitis or paracetamol overdose. ALP lines in the biliary ducts of the liver, it is also found in the bone and placental tissue. Serum ALP assessment is useful in the diagnosis of cholestatic hepatobiliary lesions and osteoblastic bone diseases [36] and also large bile duct and infiltration diseases of the liver. The response of the liver to any form of biliary duct obstruction is to synthesize more ALP.

Paracetamol–induced hepatocellular damage are often assessed by elevated serum liver marker enzymes(AST, ALT, ALP).In this study, administration of paracetamol alone significantly elevated serum levels of AST, ALT,ALT indicating hepatocellular damage, these enzymes are released into blood circulation when liver is injured[37],this result is also similar to the findings of [38]. Treatment with different fractions of *ocimum gratissimum* and silymarin resulted to decreased activities of serum liver

marker enzymes(ALP, ALT, AST),this suggest that the plant extract may have some roles in preserving structural integrity of the hepatocellular membrane ,thus prevented enzymes leakage into the blood circulation[39].

It is known that liver synthesizes a number of proteins. Hepatotoxins impair the capacity of the liver so resulting in hypoproteinemia. Hence decline in protein content can be deemed as a useful index of the severity of cellular dysfunction in liver disease. In this study, total protein level decreased significantly in group treated with paracetamol alone when compared to the control .The decreased level may be due to the impairment of the capacity of the liver by the hepatotoxins as a result of paracetamol-induced hepatotoxicity [39]. However, treatment with different fractions of ocimum gratissimum resulted into an increase in the total protein level (except ethylacetate fraction). This is a clear indication of improvement upon functional integrity of the hepatocytes and regenerated ability to synthesize proteins thereby offering protection to paracetamol-induced toxicity [40].

Bilirubin, the yellow breakdown product of normal heme catabolism is a useful tool in the assessment of hemolytic anaemia and excretory function of the liver. Elevated conjugated bilirubin indicates that the liver is conjugating bilirubin normal but is not able to excrete it [41]. Hence hyperbilirubinemia is a useful index of the severity of hepatocellular dysfunction. The findings of this work indicate that the total and conjugated bilirubin increased significantly in paracetamol treated group when compared with control. This could be an indication of biliary obstruction, when the bile ducts become blocked, bile builds up in the liver and jaundice develops due increasing level of bilirubin in the blood [42]. The significant reduction in the level of serum total and direct bilirubin following treatment with different fractions of *ocimum gratissimum* is suggestive of the hepatoprotective potential of the leaves in clearing bilirubin from the serum when it is elevated [43]The mechanism of action may be the activation of the constitutive androstane receptor (CAR), a key regulator in bilirubin clearance in the liver [44].

In the histopathological results, the normal control group of the rats did not show any histological alterations in the hepatocytes compared to paracetamol treated group that showed severe sinusoid enlargement, mononuclear infiltration, congestion and hemorrhage but the administration of different fractions of *Ocimum gratissimum* minimizes congestion, mononuclear infiltration, and cytoplasmic vacuolation of the hepatocytes caused by paracetamol intoxication.

## CONCLUSION

From this study, it can be deduced that different fractions of *Ocimum gratissimum* may exhibit hepatoprotective properties.

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Source of Support: Nil, Confl ict of Interest: None declared