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Comparative efficacy between Livolin forte® and *Ocimum gratissimum* on CCl₄ induced Liver damage in Wistar albino Rats.Ighodaro, C.N. ^{*}1, Ebo, O.E. ¹ and Agoreyo, F. ¹*Department of Physiology, College of Basic Medical Sciences, University of Benin, Edo State¹**Author for Correspondence *: chinyema@yahoo.com/+234-817-8355-708***Abstract**

In recent years, the incidence of liver disease has remained an important public health problem worldwide despite global attention to eradicate it. Both Livolin forte® (LIV) and extract of *Ocimum gratissimum* have been used separately as hepatoprotective agent. The aim of this study was to compare the efficacy of Livolin forte® and *Ocimum gratissimum* at ameliorating liver damage. Twenty-four albino Wistar rats weighing between 150-200 g were used. They were randomly distributed into six groups (6) comprising four animals in each group. Group 1 rats (normal control) received 0.3 ml/kg/day of propylene glycol, the vehicle in which LIV was dissolved, orally for 4 weeks. Group 2 (toxic) received CCl₄, dissolved in olive oil (1:1 v/v) at 0.7 ml/kg for 7 alternating days, so as to induce liver damage. Group 3 (standard) were pre-treated as group 2 after which they received oral administration of Livolin forte® at 5.2 mg/kg for 4 consecutive weeks and Group 4, 5 and 6 were pre-treated as group 2 and thereafter received oral graded doses of the extract at 100 mg/kg, 200 mg/kg and 400 mg/kg respectively for 4 consecutive weeks. The result showed that Livolin forte® group, pretreated with CCl₄ (Group 3) has a lower level of Serum Bilirubin, AST, ALT and ALP when compared with the extract of *Ocimum gratissimum* treated groups (Group 4, 5 and 6). However, this was statistically significant ($p > 0.05$). In conclusion, Livolin forte® was better as a curative agent than *Ocimum gratissimum* extract in rats. It showed a higher level of efficacy on CCl₄ induced liver damage. This supports the use of the drug for treating hepatic damage in humans.

Keywords: *Hepatoprotective, Livolin forte®*, *Ocimum gratissimum*, *Carbon tetrachloride (CCl₄)*.

Introduction

Either by way of lifestyle or job demands, Man is exposed to many hepatotoxic substances like chemicals, drugs and pesticides which leads to liver diseases such as liver cirrhosis, hepatitis and gallbladder dysfunction. In recent years, the incidence of liver disease has remained an important public health problem worldwide despite global attention to eradicate it (Subramoniam and Pushpangadan, 1999). The alteration of the liver has been considered among the deadly diseases because about 20,000 deaths and 250,000 new cases recorded each year are due to the liver disorder (Nallamilli *et al.*, 2013). The liver is the largest and one of the most vital organs in our body. It takes care of several essential physiological functions such as metabolism, secretion, and storage. In addition, it plays a crucial role in the detoxification and excretion of endogenous and exogenous substances such as xenobiotics, pharmaceutical drugs, and viral infections (Noureddine *et al.*, 2019; Jain *et al.*, 2012). However, due to these important roles, liver enzymes are used as markers in assessment of drug or plant extract safety or toxicity (Satyapal *et al.*, 2008; Tietz, 2000). Previous studies have shown that prognostic tools are used to assess liver dysfunction (Lopez-Velazquez *et al.*, 2017).

Nevertheless, in recent years a relatively new drug has been used in hospitals worldwide for the treatment and management of liver diseases. Livolin forte® contains phospholipids with

vitamins including essential phospholipids: polyunsaturated phosphatidylcholine (300mg), vitamin B1 (thiamine mononitrate, 10mg), vitamin B2 (riboflavin, 6mg), vitamin B6 (pyridoxine HCl, 10mg), vitamin B12 (cyanocobalamin, 10µg); nicotinamide (30mg) and vitamin E (alpha tocopheryl acetate, 10mg) (Olukiran *et al.*, 2014).

Although a wide range of synthetic drugs is currently employed in the management of liver dysfunction, alternative approaches from traditional medicinal system are increasingly popular in recent years. One of such medicinal plant credited with hepatoprotective activity is *Ocimum gratissimum* (Chiu *et al.*, 2012). *Ocimum gratissimum* belongs to the family *Lamiaceae* and it is also known as scent leaf ('*nchuanwu*' in igbo). It is commonly used as spice in foods. In folk medicine, *Ocimum gratissimum* is used for the treatment of fever, diarrhoea, dysentery, convulsions, pile etc. Phytochemical evaluation of this plant has shown the presence of alkaloids, phytates, tannins, flavonoids and oligosaccharides. These constituents are credited with anti-inflammatory and antioxidant properties which may be important in the hepatoprotective effect of this plant (Onaolapo *et al.*, 2011).

Generally, synthetic drugs are less readily available and relatively less affordable when compared with their plant-derived alternatives. Besides, inspirations for novel drug development can be provided by plant-derived medicines although there are experimental evidences showing that *Ocimum gratissimum* expresses liver-protecting therapeutic effects in Wistar rats, there is dearth of literature on the comparative efficacy between Livolin forte® and *Ocimum gratissimum* on CCl₄ induced Liver damage in Wistar albino Rats, hence this study. Therefore, the aim of this study was to compare the efficacy of Livolin forte® and *Ocimum gratissimum* at ameliorating liver damage.

Materials and Methods

Preparation of Aqueous Extract *Ocimum gratissimum*

Fresh leaves of *Ocimum gratissimum* were purchased from Uselu market in Benin City, Edo

State, Nigeria. It was identified and authenticated by a botanist; Pharm. F. Osuala of Department of Pharmacognosy, Madonna University, Elele Campus, Anambra state. The fresh leaves of *Ocimum gratissimum* were sorted out to remove extraneous material and rinsed under running tap water to remove debris and dust particle. They were air dried for 3 days. The dried leaves were ground into powdered form manually using a mortar and pestle. Fifty grams (50 g) of the powdered leaf was weighed out and mixed with 250 ml of distilled water; stirred carefully for about 8 minutes. This was left to stand for about 48 hours. It was then filtered using whatman filter paper and the filtrate was evaporated to dryness at room temperature in a rotary evaporator. It was stored in a refrigerator at 4 °C till required for analyses.

Experimental animals

Twenty-four (24) healthy male and female Wistar albino rats weighing 150-200 g were used in this study. The Rats were housed in wire cages, at room temperature of 29 ± 1 °C and were kept under normal environmental conditions with a 12/12h light/dark cycle. All rats were left to acclimatize for 2 weeks and their weight noted before the commencement of experimental treatment. The rats received water *ad libitum* and standard chow.

Drugs and chemicals

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and Bilirubin kits were purchased from a reputable pharmacy in Benin. Livolin forte® and Carbon tetrachloride (CCl₄) was purchased from Sigma Chemicals, USA. All chemicals and drugs used were of high quality and analytical grade.

Preparing solutions of *Ocimum gratissimum*, Livolin Forte® and administration of CCl₄

The choice of the adopted therapeutic doses was guided by the result of the oral LD₅₀ of the extract. This was taken to be less than 10% of the oral LD₅₀. Hence, doses of 100, 200 and 400 mg/kg of *Ocimum gratissimum*, adopted for this study, were prepared as follows; One gram (1 g) of the extract was weighed out and dissolved in 5 ml of distilled water to prepare a stock solution of 100 mg/ml of

Ocimum gratissimum. Stock solutions of 200 and 400 mg/ml were prepared by dissolving 2 g and 4 g of *Ocimum gratissimum* each in 5 ml of distilled water, respectively. Therefore, the rats received 0.2 ml/100 g of *Ocimum gratissimum* using an orogastric tube throughout the study period. Left-overs were stored in a deep-freezer after use and discarded after 48 h. Fresh samples were prepared every 48 h.

Each capsule of the drug (Livolin forte®) contains a total of 366 mg of LIV. A capsule was dissolved in 20 ml of propylene glycol so that 0.04 ml of the resulting solution (equivalent of 0.75 mg of Livolin Forte) was administered to a 150 g rat, orally. This is equivalent to 5.2 mg/kg, which is the therapeutic dose of the drug in humans. (Olukiran et al., 2013).

Carbon tetrachloride (CCl₄) model was used for evaluation of antihepatotoxic activity. The CCl₄ model described by Obi *et al.* (1998), was used for scheduling the dose regimen. 0.7 ml/kg intraperitoneally of CCl₄ diluted in vegetable oil (1:1) was employed for inducing liver damage.

Experimental protocol

The rats were randomly distributed into six groups (6) comprising four animals in each group. Group 1 rats (normal control) received 0.3 ml/kg/day of propylene glycol, the vehicle in which LIV was dissolved, orally for 4 weeks. Group 2 (toxic) received CCl₄, dissolved in olive oil (1:1 v/v) at 0.7 ml/kg for 7 alternating days, so as to induce liver damage. Group 3 (standard) were pre-treated as group 2 after which they received oral administration of Livolin forte® at 5.2 mg/kg for 4 consecutive weeks and Group 4, 5 and 6 were pre-treated as group 2 and thereafter received oral graded doses of the extract at 100 mg/kg, 200 mg/kg and 400 mg/kg respectively for 4 consecutive weeks. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. All conditions of animal use were in accordance with National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in strict compliance with the guidelines on Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Madonna University, Elele campus, Okija.

At the end of the study, the rats were euthanized and their blood samples were collected into separate EDTA bottles by cardiac puncture.

Biochemical Assay

Bilirubin was determined by procedure outlined in the diagnostic kit as described by Jendrassik and Grof (1938). Serum Alanine amino transferase (ALT) and Aspartate amino transferase (AST) activities were determined using a combination of the methods of Mohun and Cook (1957) and Reitman and Frankel (1957).

Measurement of body weight

Weekly body weight was assessed using Digital weighing balance (Hanson, China). The percentage weight change (PWC) was determined using the formulae below.

$$\text{PWC (\%)} = \frac{\text{Final body weight} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \times 100\%$$

Statistical analysis

Data obtained were expressed as mean \pm Standard Error of Mean using one-way analysis of variance (ANOVA) and thereafter subjected to Neuman Keuls' post-hoc test. Student's *t*-test was used to determine differences between two variables and, generally, the level of significance was set at $p < 0.05$. Data were analyzed using Graph Pad Prism 8.0 software.

Result

Effects of *Ocimum gratissimum* on percentage weight change PWC (%) of Wistar rats exposed to CCl₄ toxicity

Following CCl₄ administration, PWC was significantly lowered in the toxic group (-6.20 \pm 5.06) when compared with the control group (35.11 \pm 6.41) ($p = 0.0267$; $t = 4.350$). Also, the *Ocimum gratissimum* treated groups 4, 5 and 6 (19.07 \pm 4.65; 24.67 \pm 4.50 and 43.26 \pm 6.18 respectively) showed significantly higher PWC when compared with the toxic group (-6.20 \pm 5.06) ($p = 0.0236$; $F = 6.413$).

Effects of Livolin forte® and *Ocimum gratissimum* on Serum Bilirubin (mg/dl), AST (U/L), ALT (U/L) and ALP (U/L) levels in Wistar rats with CCl₄-induced liver injury

The results showed that Serum Bilirubin, AST, ALT and ALP levels were significantly elevated in the toxic group when compared with the

control ($p < 0.05$). The *Ocimum gratissimum* treated groups (Group 4, 5 and 6) showed a significantly lower level of Serum Bilirubin, AST, ALT and ALP when compared with the toxic group ($p < 0.05$). This reduction was in a dose-dependent manner.

Table 1: Effects of Livolin forte® *Ocimum gratissimum* on Serum Bilirubin (mg/dl), AST (U/L), ALT (U/L) and ALP (U/L) levels in Wistar rats with CCl₄-induced liver injury

GROUPS	Serum Bilirubin (mg/dl)	AST (u/l)	ALT (u/l)	ALP (u/l)
Group 1 (control)	0.53 ± 0.21	52.12 ± 2.56	40.50 ± 2.45	253.14 ± 8.13
Group 2 (Toxic Control)	0.84 ± 0.44	98.00 ± 6.57	113.31 ± 4.26	557.42 ± 2.24
Group 3 (CCl ₄ + Livolin)	0.56 ± 0.43	60.40 ± 5.45	49.20 ± 0.87	270.11 ± 3.08
Group 4 (CCl ₄ + 100 mg/kg OG)	0.80 ± 0.21	80.20 ± 2.10	62.10 ± 3.25	400.30 ± 4.32
Group 5 (CCl ₄ + 200 mg/kg OG)	0.79 ± 0.48	79.11 ± 0.24	59.30 ± 7.34	280.30 ± 5.42
Group 6 (CCl ₄ + 400 mg/kg OG)	0.66 ± 0.53	75.03 ± 3.23	52.20 ± 2.47	271.40 ± 0.23

Comparative efficacy between Livolin forte® and *Ocimum gratissimum* on Serum Bilirubin (mg/dl), AST (U/L), ALT (U/L) and ALP (U/L) levels in Wistar rats with CCl₄-induced liver injury

The livolin forte® group, pretreated with CCl₄ (Group 3), showed a lower level of Serum Bilirubin, AST, ALT and ALP when compared with the *Ocimum gratissimum* treated groups (Group 4, 5 and 6). However, this was statistically significant. Figure 1 shows the comparative efficacy between Livolin forte® and *Ocimum gratissimum* treated groups in Wistar rats with CCl₄-induced liver injury.

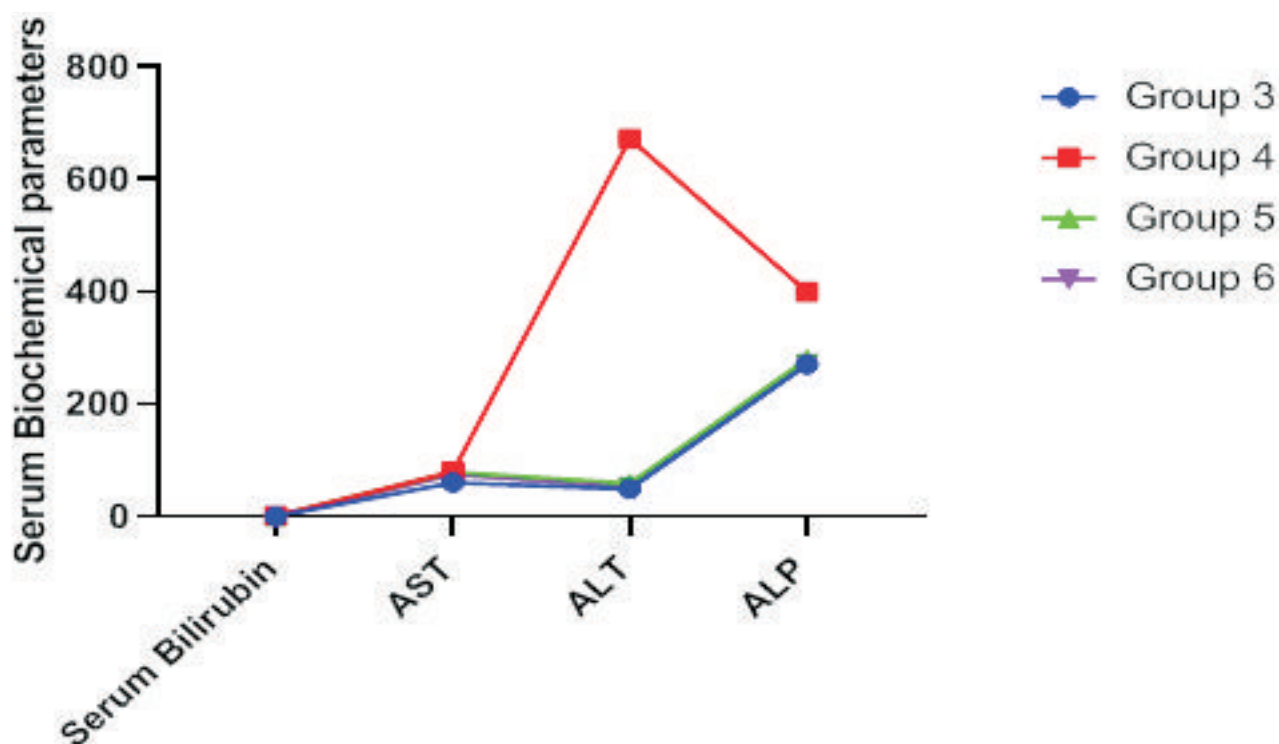


Figure 1: The comparative efficacy between Livolin forte® and *Ocimum gratissimum* treated groups in Wistar rats with CCl₄-induced liver injury

Discussion

A number of studies in the past have been conducted to investigate the hepatoprotective effects of *Ocimum gratissimum* (Arhoghro *et al.*, 2009) as well as Livolin forte® (Olukiran *et al.*, 2014) on carbon tetrachloride (CCl₄) induced liver damage in albino Wistar rats. The aim of this study was to compare the efficacy of Livolin forte® and *Ocimum gratissimum* at ameliorating liver damage. From this study, it was observed that Livolin forte® showed a lower level of the liver enzymes than all three doses of *Ocimum gratissimum* extract (Table 1). However, this reduction was statistically significant and, in a dose-dependent manner ($p > 0.05$). The significant decrease in enzyme activities of the liver Transaminase of the Livolin forte® group relative to the *Ocimum gratissimum* extract groups (Group 4, 5 and 6) could be attributed to phosphatidylcholine present in LIV. Phosphatidylcholine is known to be a class of phospholipids that protects the liver through the regeneration of liver cell (Wallnoefer and Hanusch, 1973). The significant decrease in bilirubin level in Livolin forte® group compared to the *Ocimum gratissimum* extract groups may have been as a result of enhanced bilirubin

uptake and conjugation by the regenerated liver and its subsequent secretion into the bile ducts. Thus, it could also be attributed to the presence of vitamin B₂ in this drug. Vitamin B₂ helps to maintain the integrity of red blood cells, thereby causing a decrease in haemolysis of red blood cells. Bilirubin is a by-product of red blood cells destruction (Guyton and Hall, 2001); as such, a decrease in haemolysis of red blood cells will subsequently lead to a decrease in bilirubin formation.

Also, it was observed that there may be bioequivalence between the dose 400 mg/kg of the *Ocimum gratissimum* extract and 5.2 mg/kg of Livolin forte®. This can certainly take place after a period of time due to the restoration of the normal architecture of the liver as a result of tissue regeneration.

On the other hand, there was a significantly lowered percentage weight change (PWC) that was associated with CCl₄ administration. It can be attributed to a reduction in food intake that was observed during the study period. According to Imafidon *et al.* (2005), weight gain or loss is determined by a balance between food intake and

energy expenditure. Furthermore, the observed reduction in food consumption may be as a result of the anaesthetic-mimicking effects of CCl₄, which also resulted in sluggishness of all body reflexes as well as lowered response to stimuli (Mir, 2008; Sherlock and Dooley, 1989). According to our observational study, there was continued reduction in weight of the animals regardless of its ameliorative effect on liver damage. Also, these attributes culminated in the reduction of both food consumption and body weight. Therefore, the anti-analgesic as well as appetite-stimulating effects of *Ocimum gratissimum* (in a dose-dependent manner), was also demonstrated by this study. These ameliorative effects can be attributed to the antioxidant activity of the extract. This study supports the findings of Shittu *et al.* (2016) who attributed the antidiabetic and weight loss effects of *Ocimum gratissimum* to the presence of antioxidant activity.

Conclusion

Based on our findings, we conclude that Livolin forte® was better as a curative agent than *Ocimum gratissimum* in rats. It showed a higher level of efficacy on CCl₄ induced liver damage. This supports the use of the drug for treating hepatic damage in humans. Also, the study provides experimental evidence and clearly justifies traditional claims on the use of *Ocimum gratissimum* in the treatment of liver diseases.

Conflict of Interest: Nil

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