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Research Article

Effects of Methanol Leaf Extract of *Cuphea Hyssopifolia* Kunth on Liver Enzymes Activity and Antioxidant Indices of Paracetamol-Induced Hepatotoxicity in Wistar Rats.

Moke E.G.[1*], Mordi J.C.[2], Umukoro E.K.[1]

[1]*Department of Pharmacology and Therapeutics, Delta State University, Abraka, Delta State, Nigeria.*

[2]*Department of Medical Biochemistry, Delta State University, Abraka, Delta State, Nigeria.*

ABSTRACT

The liver is constantly exposed to harmful effects of different chemical substances it detoxifies, and it is prone to inflammation which may lead to liver disease. This study investigated the methanol leaf extract of *Cuphea hyssopifolia* (CH) in preventing liver damage on paracetamol-induced hepatotoxicity in experimental rats. Twenty-five wistar rats were grouped into groups 1 and 2 which served as controls (positive and negative) receiving normal saline 10 ml/kg, groups 3 and 4 were administered 200 mg/kg and 400 mg/kg of *Cuphea hyssopifolia* leaves extract respectively, while group 5 received silymarin 100 mg/kg (standard drug treatment). Hepatotoxicity was induced with 3 g/kg of paracetamol (PCM) on the 7th day in all the animal groups except the positive control group (Group 1). At the end of the experiment, blood samples were collected for biochemical indices (liver enzymes and antioxidants) and liver tissue for histology. CH significantly ($p < 0.05$) reduced the raised liver enzymes level, and also increased serum levels of superoxide dismutase and catalase, while reducing lipid peroxidation level via a decrease in serum malondialdehyde measure when compared with the PCM-treated (negative) control group. The findings revealed that the methanol leaf extract of *Cuphea hyssopifolia* possesses potent hepatoprotective activity.

Keywords*: antioxidant, hepatoprotective, Cuphea hyssopifolia, paracetamol, malondialdehyde*

*Author for correspondence: Email: *hiligoodies@gmail.com*; Tel: *+234-7061040692*

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INTRODUCTION

Herbal medicine (phytomedicine) refers to using plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. Advances in clinical research, analysis, and quality control has shown the value of herbal medicine in treating and preventing disease, and it has become a mainstay (Altschuler, *et al.*, 2007; Tungmunnithum *et al.*, 2018). From ancient time to the present days, all cultures have used plants as a source of medicine (Elujoba, 2005). The World Health Organization (WHO) reports that traditional medicine including herbal drugs have been used for many centuries in many parts of the world for health care (WHO, 2010). Some of these herbal plant extracts exhibit therapeutic properties while others have been found to be harmful due to the nature of their constituents (Wu *et al*., 2006; Oladeji, 2016).

Cuphea hyssopifolia Kunth (Lythraceae), a small shrub, is the largest of the thirty-two genera of Lythraceae with about 260 species of herbaceous perennials and small shrubs (Graham, 2017). A wide range of phytochemical constituents have been reported from genus Cuphea viz. tannins, flavonoids, phenolic acids, triterpenes, sterols, carbohydrates and unsaturated fatty acids (Elgindi *et al.*, 2011). Various biological activities have been reported, which include its cytotoxic, antiviral, antimicrobial, antiprotozoal, cardiovascular, antioxidant and diuretic activities (Elgindi *et al.*, 2011).

The liver is a vital organ that plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis hormone production, and detoxification (Adewusi and Afolayan, 2010). The liver's highly specialized tissues regulate a wide variety of high volume biochemical reactions, including the synthesis and

breakdown of small and complex molecules, many of which are necessary for the normal vital functions. Hepatic disease indicates damage to the cells, tissues, structure, or function of the liver, which may be induced by toxic compounds (including drugs) as well as other biological factors (Amengual-Guedan and Rodriguez-Sanchez, 2000; Casafront-Morencos *et al.*, 2008).

Due to the exposure of the liver to chemicals, drugs and even food that may be toxic to it, there is need to research on drugs (of herbal origin) that could help to reverse hepatotoxicity and protect the liver. Thus, the purpose of this work using experimental rat.

MATERIALS AND METHODS

Plants: Fresh leaves of *Cuphea hyssopifolia* were collected from the school environment and were authenticated by a taxonomist in the Department of Botany, Faculty of Sciences, Delta State University, Abraka, Nigeria.

Preparation of plant extract: Dried leaves of *Cuphea hyssopifolia* were powdered with the aid of a mechanical grinder. Seventy-eight gram (78 g) of the powdered leaf was dissolved in 1400 ml of 70% methanol and extracted using Soxhlet evaporator at 25-35^{0}C. The filtrate was further concentrated to dryness with the aid of a water bath set as 45-50^{0}C. The weight of the final extract was recorded and stored in the refrigerator prior to the study.

Animals: Twenty-five (25) adult Wistar rats weighing between 100-125 g were procured from the Animal House of the Faculty of Basic Medical Science, Delta State University Abraka, Nigeria. The animals were acclimatized for a period of two weeks prior to the study, and were placed on Growers' feed and clean water *ad libitum*. Guidelines followed in the handling of animals were in accordance with the ethical standards of the Institutional Animals Ethics (IAEC), as adopted by the ethical committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria.

Drugs: Paracetamol (PCM) 500 mg tablet was obtained from Emzor Pharmaceuticals (Nigeria) while the Silymarin (Silybon®) 100 mg tablet used was manufactured by; Micronova, India).

Paracetamol-induced hepatotoxicity: The rats were randomly placed into 5 groups of 5 animals each as follows:
Group 1 - Normal Saline 10 ml/kg
Group 2 - Normal Saline 10 ml/kg + PCM 3000 mg/kg (7th Day)
Group 3 - *Cuphea hyssopifolia* 200 mg/kg + PCM 3000 mg/kg (7th Day)
Group 4 - *Cuphea hyssopifolia* 400 mg/kg + PCM 3000 mg/kg (7th Day)
Group 5 - Silymarin 100 mg/kg + PCM 3000 mg/kg (7th Day)
The experimental animals were administered the extracts orally daily for 6 days according to their body weights. All the animals except the normal control group (Group 1) were administered PCM 3000 mg/kg on day 7 and then observed for 24 hours before they were sacrificed for sample collection. The animals were subjected to chloroform anaesthesia and blood samples were drawn, allowed to clot, centrifuged and serum collected to analyze for the biochemical indices. The liver of each animal was harvested for histopathological studies.

Determination of liver enzymes function test Alkaline phosphatase (ALP), aspartate aminotransefrase (AST), and alanine aminotransferase (ALT) in serum were determined according to methods described by Reitman and Frankel (1957) and Roy (1970).

Determination of antioxidants activity: Superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels were analyzed using methods of Misra and Fredovich (1972), Sinha (1972), and Gutteridge and Wilkins (1982).

Histopathology: Sections of liver samples fixed with formalin, were stained with haematoxylin-eosin for photomicroscopic observations of the liver histological architecture.

Data analyses: Results were presented as mean ± standard error of mean (SEM) using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P-values < 0.05 were taken as significant.

RESULTS

Oral administration of PCM resulted in significant ($p < 0.05$) elevation in the levels of AST, ALT, and ALP in the toxin control group when compared with the normal control group (Table 1). Treatment of the animals with both 200 and 400 mg/kg of CH extract orally prior to PCM administration resulted in reversal towards normal of the levels of these serum marker enzymes.

Table 1:
The effect of methanol leaf extract of *Cuphea hyssopifolia* (CH) on liver enzymes function test in paracetamol-induced hepatotoxicity

	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal control	46.41 ± 1.51	15.53 ± 1.80	31.23 ± 3.27
PCM control	69.72 ± 0.94	26.92 ± 3.18	55.77 ± 1.18
CH 200 mg/k + PCM	49.38± 1.27*	13.94 ± 2.38*	35.74± 2.06*
CH 400 mg/kg + PCM	49.26± 0.48*	12.26 ± 2.32*	39.21± 2.30*
Silymarin 100 mg/kg + PCM	50.86± 0.75*	13.94 ± 1.85*	40.42± 2.04*

*All values expressed as Mean ± SEM, where n=5, all data were analyzed by using one-way ANOVA followed by Tukey's post hoc test. *=P<0.05 was taken to be significant when compared with the PCM control.*

Treatment with silymarin 100 mg/kg (standard drug) had the same significant ($p < 0.05$) decrease in the enzymes level. The observed hepatoprotective effect of the extracts of CH is also supported by the histopathological findings revealing an improved state of the liver of animals treated with CH (Plate 1).

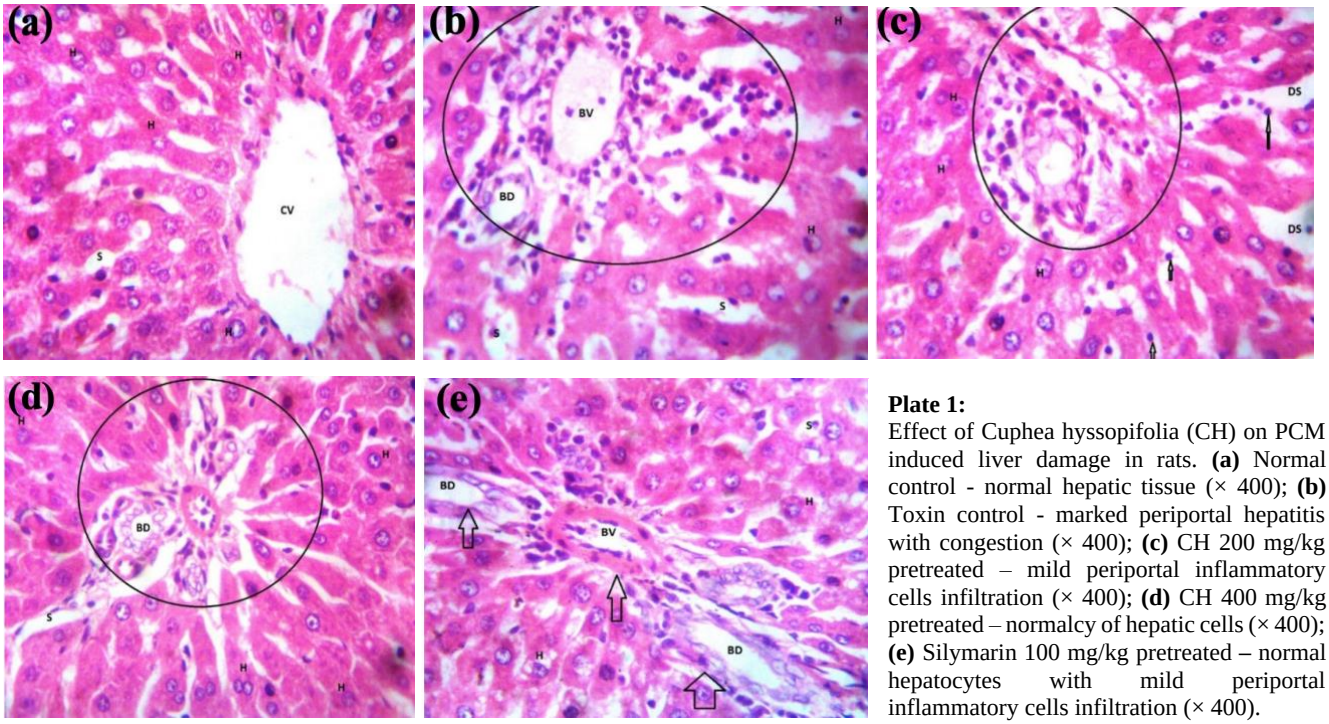

Plate 1:
Effect of Cuphea hyssopifolia (CH) on PCM induced liver damage in rats. **(a)** Normal control - normal hepatic tissue (× 400); **(b)** Toxin control - marked periportal hepatitis with congestion (× 400); **(c)** CH 200 mg/kg pretreated – mild periportal inflammatory cells infiltration (× 400); **(d)** CH 400 mg/kg pretreated – normalcy of hepatic cells (× 400); **(e)** Silymarin 100 mg/kg pretreated **–** normal hepatocytes with mild periportal inflammatory cells infiltration (× 400).

Administration of PCM caused significant ($p < 0.05$) decrease in superoxide dismutase (SOD) and catalase (CAT) levels, and a significant increase in lipid peroxidation level via increase in MDA in the rats when compared with normal control animals (Table 2). Pretreatment with CH extracts showed significant ($p < 0.05$) increase in CAT level at both doses of 200 and 400 mg/kg, and a non-significant ($p > 0.05$) increase in SOD at 400 mg/kg. A much significant reduction of the lipid peroxidation level was seen with the pretreated CH rats at both doses when compared with the PCM treated-only rats (toxin group).

Table 2:
The effect of methanol leaf extract of *Cuphea hyssopifolia* (CH) on antioxidant indices in paracetamol-induced hepatotoxicity

	SOD (IU/L)	CAT (IU/L)	MDA (IU/L)
Normal control	0.31 ± 0.03	0.52 ± 0.06	0.58 ± 0.06
PCM control	0.27 ± 0.06	0.45 ± 0.09	0.72 ± 0.07
CH 200 mg/k + PCM	0.25 ± 0.02	1.32 ± 0.06*	0.45± 0.02*
CH 400 mg/kg + PCM	0.32 ± 0.01	1.80 ± 0.01*	0.45 ± 0.04*
Silymarin 100 mg/kg + PCM	1.00 ± 0.51*	1.51 ± 0.04*	0.42 ± 0.05*

*All values expressed as Mean ± SEM, where n=5, all data were analyzed by using one-way ANOVA followed by Tukey's post hoc test. *=P<0.05 was taken to be significant when compared with the PCM control.*

DISCUSSION

New drugs of herbal origin are being developed for treatment of hepatitis (Adetutu and Owoade, 2013). Paracetamol (an antipyretic) has been used to induce liver damage since it is a well-known hepatotoxin at very high doses. Necrosis of hepatocytes by paracetamol (PCM) is observed following an increase in levels of serum transaminases (Vermeulen, 2012). This present study investigated the ability of methanol leaf extract of *Cuphea hyssopifolia* (CH) at 200 and 400 mg/kg in decreasing the extent of liver toxicity caused by PCM.

Decrease in the activity of CAT due to exhaustion of the enzyme as a result of the oxidative stress induced by PCM (Jia *et al.*, 2011), was brought to normalcy and better improved in states of pretreatment with CH. Also, both doses of CH used in this study effectively decrease the extent of lipid peroxidation caused by PCM liver damage. Thus, CH has free radical scavenging activity and hepatic tissue healing property. *Cuphea hyssopifolia* has been reported to have high phytochemical contents of flavonoids, phenols, and tannins, which have aided its antioxidant and cytotoxic actions (Schuldt *et al.*, 2004; Elgindi *et al.*, 2012; Tungmunnithum *et al.*, 2018; Masoko and Masiphephethu, 2019).

In conclusion, the findings of this study shows that the methanol leaf extract of *Cuphea hyssopifolia* possesses potent hepatoprotective activity. Hence, can be used in traditional medicine for the treatment of hepatic disease and protection against toxic effect of chemical substances

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