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Crassocephalum crepidioides (Ebolo) Protect against Paracetamol-Induced Hepatotoxicity in Wistar Rats

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Abstract- Paracetamol causes hepatocellular damage due to increase oxidative stress, whereas *Crassocephalum crepidioides* enhances antioxidant status. The preventive function of *Crassocephalum crepidioides* against paracetamol (PCM)-induced hepatocellular oxidative damage was studied. The experiment was made up of four groups (1, 2, 3, and 4) of Wistar rats (n=6). The control (Group 1) were given distilled water while groups 2 and 3 animals were given 300mg/kg bwt/day methanol extract of *C. crepidioides* leaves (MECL) and 250 mg/kg bwt/day PCM respectively for two weeks. Group 4 was given (MECL) for one week and thereafter a concurrent dose of 300 mg per kg bwt each day (MECL) and 250 per kg bwt each day PCM for two weeks. All administration was done orally. The evaluation of serum aspartate amino transferase (AST) and Alanine amino transferase (ALT) activities were carried out to detect liver injury. Glutataione-s-transferase (GST), superoxide dismutase (SOD), catalase activities and reduced glutathione (GSH) level were utilized to measure antioxidant status. Malondialdehyde (MDA) level was employed as an indication of lipid peroxidation. The results indicated an increase ($p< 0.05$) in the activities of serum AST, ALT and MDA level in the PCM group. The activities of GST, SOD, catalase and level of GSH were significantly lowered ($p< 0.05$) relative to control. Concurrent treatment with PCM and *C. crepidioides* considerably decreased ($p< 0.05$) MDA level, AST and ALT activities but significantly increased ($p< 0.05$) the antioxidant enzymes compared to PCM group. Finally, *Crassocephalum crepidioides* may protect Wistar rat's liver against paracetamol-induced oxidative damage.

Keywords **–**Antioxidant, *Crassocephalum crepidioides*, Liver damage, Paracetamol

I. INTRODUCTION

The liver is identified as the body's largest and most complicated organ. It regulates a variety of functions including metabolism, secretion, and storage. In most cases, the liver's metabolism of endogenous and foreign substances results in their detoxification and excretion. Oxidation, reduction, hydration, hydrolysis, conjugation, and isomerization are all methods used by the human liver to metabolize chemicals. Hepatotoxicity can occur if these systems are disrupted [1]. Most of these physiological activities are disrupted when the liver is injured [2]. Abrupt liver failure is mostly due to medication-induced hepatotoxicity, and paracetamol (PCM), scientifically also referred to as acetyl-para-aminophenol (APAP) or acetaminophen causes hepatotoxic. [3]. It's also one of the most often prescribed analgesics and antipyretics in the world, and it's available over-the-counter in mono- and multi-component formulations [4], making it easily accessible to individuals. This, along with its limited therapeutic index, makes overdosing a regular occurrence. Although, when used in therapeutic levels, it is a safe medicine.

At therapeutic doses, paracetamol is rapidly converted to inactive metabolites by the enzymes glucanosyltransferases and sulfotransferases, which conjugate it with glucuronic acid or sulphate, respectively. Cytochrome-p450 (CYP450) processes the metabolite; N-Acetyl-p-benzoquinoneimine (NAPQI), that is produced through the minor route for paracetamol metabolism. In humans and experimental animals NAPQI induces hepatic damage [5] and renal tubular necrosis [6]. It is detoxified by combining glutathione with cysteine and mercapturic acid to generate mercapturic acid and cysteine conjugates; consequently, eliminated via the kidneys [7]. However, when this route is exhausted, such as in acetaminophen overdose or glutathione shortage, NAPQI metabolism is reduced, thus, NAPQI poisoning occurs due to an overdose or prolong use. Paracetamol overdosing or its long-term use causes glutathione depletion, resulting in an imbalance between antioxidants and free radicals in the body, and thus oxidative stress. NAPQI also interacts with cellular membrane molecules, forming covalent adducts, thereby causing hepatotoxicity [8].

For local customers, green leafy vegetables are a major source of vitamins, minerals, and fiber [9]. Many scientific investigations on their potentials have been conducted due to their nutritional relevance. Several researchers have studied various plants with medicinal properties in a bid to determine the feasibility of developing affordable, sustainable and natural drugs [10], after concluding that some vegetables and fruits contain phytochemicals with antioxidant properties.

Crassocephalum crepidioides, referred to as 'Ebolo' in Western Nigeria, is an herb which grows up to 180 cm tall and is slightly succulent. It is notably popular in tropical Africa, where the leaves are consumed as vegetables [11]. Phytochemicals (phenol, flavonoids, tannin and alkaloids) were found in the leaves of *C. crepidioides* [12]. MECL has anti-inflammatory and antioxidant action, suggesting that it might be used to treat PCM-induced hepatotoxicity [13].

Paracetamol is a well-tolerated medicine that is available over-the-counter, which means that it may be purchased without a doctor's prescription. However, as a result of its toxicity, the number of instances of paracetamol-induced liver poisoning has progressively increased over the world [4]. In both people and animals, paracetamol overdose is treated with N-acetylcysteine (NAC) (antidote for APAP-induced hepatotoxicity). This drug has limited effect against APAP-induced hepatotoxicity and can be depleted [14]. As a result, finding a more efficient drug that will be effective in combating the toxicity of APAP is critical [15]. The growing interest in the pharmacological potentials of herbs, vegetables and fruits as antioxidants in decreasing free radical related diseases made them important for production of new drugs. The phytochemicals in plants possess antioxidant activities that can combat ROS generation and thus can be useful in preventing the effect of PCM toxicity since the mechanism of PCM toxicity is related to the formation of ROS and decreased antioxidant level. The search for an alternate drug for the treatment and prevention of PCM toxicity can therefore be met in plant such as *Crassocephalum crepidioides* with excellent antioxidant activities. This plant also possesses hepatoprotective effect against carbontetrachloride (CCl_4) liver damage [16]. Hence, this study seeks to confirm the hepatotoxic effect of PCM and evaluate the protective effect of *Crassocephalum crepidioides* leaves on PCM-induced hepatotoxicity.

II. RELATED WORK

The liver regulates biochemical and physiological functions, including xenobiotics and drug detoxification [17]. The PCM reactive metabolite NAPQI has been found to initiate PCM-induced hepatotoxicity by formation of ROS [17], [18]. In addition, Mahmood et al. [19] discovered that PCM overdose elevated serum ALP, AST, and ALT activities, while Wicaksono et al. [20] discovered that rats treated with paracetamol and morphine had an increase in ALT and AST.

Medicinal herbs have long been recognized as a valuable source of therapies or curative aids, and they contain chemicals, such as tannins, flavonoids, alkaloids, and terpernoids, which determine plant's medicinal effectiveness [21].

Tannins, mucilage, coumarins, flavonoids, steroids, proanthocyanidin and reducing chemicals, and have been found in *C. crepidioides* leaves [22]. Indigestion, hepatic insufficiency, and intestinal worms have all been treated with it. The herb has strong antioxidant properties and can protect the liver against poisoning [23]. *C. crepidioides* was shown to protect against hepatotoxicity and cellular damage caused by CCl_4 [24]. *C. crepidioides* leaf reduced the activities of plasma indicators of liver damage, such as ALT, AST and GGT as well as increased catalase and SOD activities, with a drop in MDA levels, indicating that the leaf can protect against oxidative damage.

III. MATERIALS AND METHODS

Collection of plant and Extract Preparation.
Fresh *Crassocephalum crepidioides* (Ebolo) leaves were purchased at Ilara-Mokin, Akure, Ondo State, and verified at the University of Ibadan's Department of Botany. The leaves were then cleaned under running water, divided into smaller portions, and air dried at room temperature; away from direct sunlight. The dry samples were ground into a fine powder and weighed to increase surface area.

Preparation of Aqueous (Methanol) Extract of *Crassocephalum crepidioides*.
To obtain the extract, a dried sample of *C. crepidioides* leaves weighing 333g was mixed and cold macerated (soaked) in 2500mls of 70% methanol in a Winchester bottle for 72 hours. This was filtered and dried in a rotatory evaporator and kept in the fridge until it was needed. The percentage yield of the leaves is 7.56%.

Research Animals
Wistar rats were procured from the Animal House, Department of Physiology, Federal University of Technology, Akure, Nigeria. The rats were housed in netted cages under standard laboratory conditions and were fed with standard rat's pallets and tap water provided ad-libitum. Excess feed and water were removed and replaced daily. The rats were allowed to acclimatize for 2 weeks before the commencement of the experiment. Thereafter, the rats were treated as follows. The study followed the International Ethical Norms on the care of Animals and Use, as published in NIH publication/80-23, which was amended in 2010.

Treatments for Animals
24 rats (180-200g) were separated into four groups (1,2,3 and 4). Each group consisted of six rats each. Distilled water was given to the control group (Group 1) for two weeks. Group 2 and 3 animals were given 300 mg MECL per kg body weight (bwt) per day and 250 mg PCM per kg bwt per day, respectively. Animals in group 4 were given

MECL orally for 1 week before receiving a concurrent treatment of 300 mg MECL per kg body weight (bwt) per day and 250 mg PCM per kg bwt per day for 2 weeks. Animals were examined on a daily basis and weighed every three days for two weeks. They were also kept on their diets throughout this time. Before being killed via cervical dislocation, the animals were removed from therapy for 24 hours and starved for 12 hours.

Preparation of Rat Post-Mitochondrial Fractions of Liver and Blood Sample

Blood sample was obtained through heart puncture for biochemical analysis. To make the serum, blood was collected into sample tubes and centrifuged for 10 minutes at 3000g. After sacrifice; liver was removed and cleaned in 1.15 percent KCl solution at 0^o C. The liver was homogenised in a 1:3 ratio of sodium phosphate buffer at a pH of 7.4 and centrifuged for a period of 10 minutes at 10,000xg to extract the post-mitochondria fraction for the analysis of enzymes.

Biochemical Analysis (Determination of Organ Damage and Antioxidant Status).

Organ damage was assessed by evaluation of blood AST using the Randox AST kit and blood ALT using Randox ALT kits. The antioxidant status was assessed by determination of the lipid peroxidation level, the antioxidant enzymes activities; superoxide dismutase (SOD), GST and Catalase and the level of the non-enzymatic antioxidant; Glutathione (GSH).

Determination of Lipid peroxidation

Lipid peroxidation was evaluated according to the method described by Buege and Aust [25]. The method is based on the reaction between thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxide during peroxidation.

Determination of Superoxide dismutase activities

Superoxide dismutase (SOD) activities was determined as described by Misra and Fridovich [26]. The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for the dismutase.

Determination of GSH and GST activities

*T*he activity of glutathione-S-transferase in serum was determined according to the method of Habig *et al.* [27]. Glutathione-S-transferase demonstrates a relatively high activity with 1-chloro-2,4-dinitrobenzene as the second substrate; consequently, the conventional assay for glutathione-S-transferase activity utilizes 1-chloro-2,4-dinitrobenzene as substrate. The method of Beutler *et.al.* [28], was followed in estimating the level of reduced glutathione (GSH). The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow color when 5,5 – dithiobis – (2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds.

Determination of catalase activities

Catalase activities was assessed according to the method of Sinha [29]. This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate produced is measured colorimetrically at 570-610 nm.

Statistical Result Analysis

The results expressed are Mean ± SEM. Data analysis was done by the one-way analysis of variance (ANOVA) and the LSD post hoc test by Fischer. Differences among groups were considered to be significant at a P-value of <0.05. All analyses were done by using GraphPad Prism version 8.0 (GraphPad® Inc, CA, USA).

IV. RESULTS AND DISCUSSION

Results

Significant increase was observed in ALT and AST levels in the group orally administered 250 mg/ kg bwt/day PCM compared with the control as shown in Table 1. Co-administration of the extract with PCM attenuated the AST activity significantly ($p<0.05$). ALT activity was slightly attenuated by the co-administration of the extract since the ALT activity for the group on PCM + extract was lowered significantly ($p<0.05$) when compared with the PCM treatment but was relatively higher ($p<0.05$) than that obtained for group 1.

Table 1**:** MECL's effect on Markers of Liver Damage During PCM-induced Hepatotoxicity in Rats

	ALT activity (u/L))	AST activity (u/L)
G1 (Control)	9.46±0.14	48.00±1.15
G2 (Ebolo extract)	9.93±0.17$^{\#}$	45.26±1.20$^{\#}$
G3 (PCM 250mg)	15.42±0.18*	87.58±0.90*
G4 (PCM + Extract)	10.31±0.15*$^{\#}$	47.26±1.17$^{\#}$

Values represent mean ± SEM. *$p<0.05$ shows that the group is different significantly compared with the control; # $p<0.05$ shows that the group is different significantly compared with the PCM.

The result for GSH level and GST activity in table 2 showed that GSH level and GST activity were reduced significantly in the animals treated with 250 mg PCM per kg bwt per day compared to control. Administration of *Crassocephalum crepidioides* with PCM significantly increased the activities of these enzymes ($p<0.05$). Also represented in this table is the significant reduction in SOD and catalase activities ($p<0.05$) in animals given 250 mg PCM per kg bwt per day when compared with the control. Concurrent administration of *C. crepidioides* extract significantly increased SOD and catalase activities ($p<0.05$) reletively to the PCM only treated group.

Table 2: MECL's effect on the Antioxidant Levels in Paracetamol-induced Hepatotoxicity in Rats

	GSH levels(µg/ml)	GST activities (µmole/min/mg protein)	SOD activities (U/mg)	Catalase Activities (Katf)
G1 (Control)	0.011±0.00023	0.21±0.012	10.90±0.40	0.0831±0.0029
G2 (Ebolo extract)	0.012±0.00028*#	0.25±0.007*#	12.12±0.45*#	0.1052±0.0025*#
G3 (PCM 250mg)	0.005±0.00063*	0.18±0.031*	5.80±0.49*	0.0346±0.0014*
G4 (PCM + Extract)	0.010±0.00028*#	0.21±0.012*#	8.20±0.2*#	0.0754±0.0042*#

Values represent mean ± SEM. *p<0.05 shows that the group is different significantly compared with the control; #p<0.05 shows that the group is different significantly compared with the PCM

As expressed in figure 1 the MDA level of animals given 250 mg/ kg bwt/day PCM was higher significantly when compared with the control. This was lowered significantly in the presence of *C. crepidioides* extract

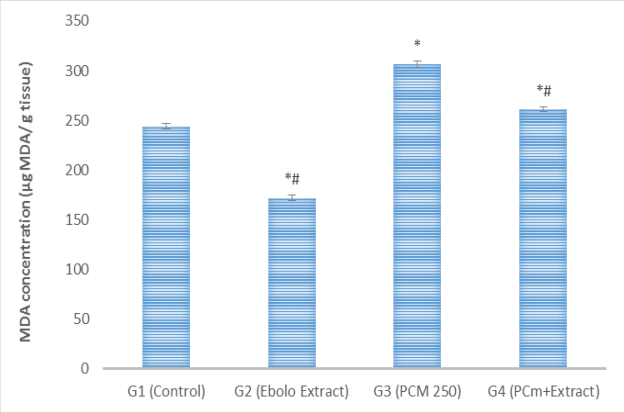

Figure 1: MECL's effect on the level of malondialdehyde in paracetamol-induced hepatotoxicity in rats. Bars are means ± SEM *p<0.05 shows that the group is different significantly from the control; #p<0.05 shows that the group is different significantly from the PCM

Discussion

Liver plays a crucial function in avoiding compound buildup by transforming them into forms that can be easily excretion. In general, drug intermediate products have the potential to harm the liver. PCM is a powerful antipyretic medication that acts in the central nervous system to block the production of prostaglandin [30,31,32]. It is also used as an and analgesic. Higher than therapeutic dosages can produce toxicity, with adverse effects including liver damage [33]. Free radical that include reactive oxygen/nitrogen species are shown to be involve in the mechanism of paracetamol toxicity. *Crassocephalum crepidioides* are rich in phytochemical such as polyphenol which are of health benefits in preventing diseases and oxidative damage to organs because they are good scavengers of free radicals [12, 16].

In this investigation, ALT and AST activities in the serum substantially increased in the group that received oral doses of 250 mg/kg bwt/day PCM. ALT and AST are enzymes found in the cytoplasm and mitochondria of cells, respectively [34]. The spilling of these cellular enzymes into the blood is caused by hepatic cell destruction [35]. Thus, increased serum liver enzyme values in the group that got 250 mg/kg bwt/day PCM indicates hepatocyte injury. *Crassocephalum crepidioides* leaf extract has also been demonstrated to reduce blood ALT and AST activity in mice during rifampicin-induced oxidative stress [35], whereas PCM has been proven to produce hepatotoxicity in rats with high ALT and AST levels in the blood [36].

The active thiol group that functions as an antioxidant is carried by GSH. This group interrelates with reactive oxygen/nitrogen species and detoxify both endogenous and external electrophilic poisons. Some ROS species can be bound directly by GSH, whereas others can use GSH as a reductive power source. GST catalyses the detoxification of electrophiles by GSH [37]. The activity of reduced glutathione (GSH) was estimated and our findings showed that this was drastically lowered in the group that received in the PCM alone. Acetaminophen is metabolized to toxic intermediate metabolites such as NAPQI in the liver by CYP450. Detoxification of NAPQI occurs by its conjugation with GSH. At higher doses of PCM, GSH levels may be depleted, thus accumulating NAPQI, which results in toxicity by covalently binding with the sulfhydryl groups of protein to form protein adduct [38]. As a result, the lower GSH level detected in this study may be caused by its greater usage in the metabolism of large doses of PCM. This event causes liver cell damage. GSH deficiency is linked to a variety of liver disorders, including alcoholic steatohepatitis, nonalcoholic steatohepatitis, and liver cirrhosis [32]. Our result indicated that although GSH level was not increased significantly in animals administered *C. crepidioides* with PCM relative to the control, but was only increased relative to the animals administered only PCM. However, GSH was increased in the group that received only *C. crepidioides* (Ebolo) extract relatively to the control, indicating its ability to increase the antioxidant status in animals. *C. crepidioides* is rich in antioxidant [39] and have been reported to prevent myocardiac infarction by increasing the GSH level and other antioxidant enzymes [40].

Glutathione transferases (GSTs) are enzymes that biotransform electrophilic substances. In this approach, GSTs protect organisms from both external (xenobiotics) and endogenous (carcinogenic) chemicals. GST detoxify electrophilic chemicals by catalyzing GSH conjugation with these molecules, while some also catalyze lipid peroxide reduction [41]. When APAP is oxidized in the presence of CYP450, electrophilic intermediates are formed that can be processed by GST [8]. The activity of GST was considerably lowered during PCM treatment relatively to the control. However, concomitant administration of *C. crepidioides* extract substantially boosted GST activity. GST's decreased activity in the PCM group might be attributed to its depletion in the conversion of the toxic molecule (NAPQI) by GSH into a non-toxic product. This situation may allow unconjugated NAPQI to attach to macromolecules in cells, leading to hepatotoxicity [8]. Glutathione is essential for cell defense

against ROS and its deficiency results in a drastically decreased cellular state. Furthermore, the intracellular level of GST is a useful biomarker for monitoring tissue damage, thus the reduced GSH and GST observed for the PCM group is an indication of a reduction in the body's defense system against ROS [42]. *C. crepidioides* may therefore reduce harmful chemical buildup in the liver by raising GST activity and GSH level, indicating that the plant may have hepatoprotective properties. Similar study also showed the hepatoprotective effect of *C. crepidioides* with increased GSH level during oxidative stress induced by rifampicin [35]

Superoxide anion is transformed to oxygen molecule and hydrogen peroxide (H_2O_2) by SOD [43], whereas catalase converts H_2O_2 to water and molecular oxygen, reducing the harmful consequences of hydrogen peroxide, which creates the very toxic hydroxyl radical via the Fenton's reaction. [44]. PCM have been reported to decrease the activities of the antioxidants SOD and catalase as well as the level of GSH [36]. Decrease in SOD and catalase activities in the 250 mg/ kg bwt/day PCM only administered group when compared to the control was observed; this was only slightly improvement in the presence of the extract because co-administration of the extract significantly increased SOD and catalase activities relative to the 250 mg/ kg bwt/day PCM only administered group and were significantly lowered relative to the control. Earlier studies have shown that *C. crepidioides* prevented myocardial infarction with an increased SOD and catalase activities [40]. Decreased in the activity of SOD and catalase have been linked to various human health issues; conversely, therapy with these antioxidant enzymes has been demonstrated to alleviate liver oxidative stress in animals [43], [44]. As a result, the capacity of the *C. crepidioides* extract to boost the SOD and catalase activities of the animal during PCM poisoning may protect against liver damage.

The increase in the amount of MDA expresses oxidative stress. It is caused by a relatively high quantity of ROS and/or a decline in the antioxidant defense system against ROS [45], and it has been linked to the pathogenesis of various neurodegenerative illnesses. The treatment with 250 mg/kg bwt/day PCM raised the level of SOD in the animals, according to our findings. The co-administration of *C. crepidioides* attenuated this impact, showing the plant's potential to alleviate oxidative stress. Tripathi et al. [46] found that PCM decreases anti-oxidant indicators in the liver by decreasing SOD, catalase, GST, and GSH levels and increasing MDA levels. Thus, the PCM-induced hepatotoxicity seen in this study might be caused by the reduced GSH level and antioxidant enzymes mentioned above, which could lead to oxidative stress. Hota *et al.* [36] also reported the hepatotoxicity effect of PCM with a decrease in the antioxidant SOD, GSH and catalase. Previous research has revealed that *C. crepidioides* contains active chemicals such as flavonoids, oxalate, phenolics, phytate, saponins, and tannins [47], as well as antioxidant activity [48]. The antioxidant action of *C. crepidioides* is attributed to its high phytochemical content. Furthermore, the plant's capacity to reduce oxidative stress, as evidenced by reduced MDA levels in *C. crepidioides* co-administered mice, may be due to its influence on raising the level of non-enzymatic antioxidant GSH, activities of catalase, SOD and GST. This increase in antioxidant capability may result in a reduction in ROS levels. These findings are consistent with previous research which showed that the extract reduces oxidative stress in an Alzheimer's disease induced fruit fly by raising the levels of SOD, catalase, GST, and GSH [42].

V. CONCLUSION AND FURTHER STUDIES

Finally, in the liver of wistar rats PCM was shown to cause oxidative stress; although this m may not result in liver damage as there were no change in the evaluated markers of liver damage. This suggest that the oxidative stress is in the early stage. However, *C. crepidioides* may protect the liver of Wistar rats from paracetamol-induced oxidative stress by raising the amount of antioxidant enzymes.

Further research will be conducted to find the active chemicals in *C. crepidioides* that contribute to its high antioxidant content. This is especially important in identifying compounds that can reduce GSH and GST levels in animals in order to build highly safe nutritional supplements and medications that can be used to prevent and cure paracetamol toxicity, respectively. Histological tests will be conducted as well to establish the effect of paracetamol on liver architecture.

Conflicts of Interest

The authors state that there are no conflicts of interest.

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