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Healing potentials of mixture of *Zingiber officinale*, *Curcuma domestica and Garcinia kola* extracts on Renal and Hepatic tissues of Rats pre-treated with Cyclophosphamide

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ABSTRACT

Background: *Zingiber officinale, Curcuma domestica, and Garcinia kola* have been a regular feature in herbal medicine as typical feedstocks. They are mainly used as antibiotics and anti-inflammatory agents. This research work was designed to further investigate ethnobotanical uses of these plants to ascertain their ameliorative roles the on cyclophosphamide effect on histopathological and biochemical indices of renal and hepatic tissues of albino rats.

Methods: The study used 40 rats in all and was done in two phases. Two groups were created for phase 1, group 1 (n = 6) fed with rat feed and water served as the control group; group 2, (34 rats) was given 10 mg/kg of cyclophosphamide. The animals were weighed, anesthetized, and 6 of the 34 rats (in group 2) were randomly selected and sacrificed after 5 weeks; blood samples, livers and kidneys were examined histologically. In phase 2, the remaining 28 rats were divided into seven groups (A, B, C, D, E, F, and G, n = 4) and treated with different doses of individual and combined extracts of the plants. After six weeks, the blood samples, livers and kidneys of all the animals were histologically analyzed.

Results: There were significant ($P< 0.05$) increases in aspartate aminotransferase, alkaline phosphatase, and alanine transaminase as well as degenerative effects on the liver and kidney, which were significantly decreased by treatment with different combinations of the extracts.

Conclusion: The plants extract could serve as remedial treatment renal and hepatic problems as well as healthy food for mankind.

***Key words*— Cyclophosphamide, Ginger, Bitter kola, Turmeric**

1. INTRODUCTION

The use of herbal medicine is widespread and widely accepted in Africa, including Nigeria, an African nation. Nigeria is a country with three main ethnic groups, and each of these groups uses herbal medicine and has local names for various plants based on their functions and uses. The Hausa people refer to ginger as Ata-ile. Igbo are known as Chita. The Hausa call turmeric "Gangamau," while the Yoruba call it "Jinja." The Igbo refer to bitter kola and ata-ile pupa as aku ilu, Hausa refer to it as namijin goro, and Youruba refer to it as orogbo. In Nigeria, these three herbal plants—*Zingiber officinale, Curcuma domestica, and Garcinia kola*—are used for a variety of purposes [1]. *Garcinia kola* (Bitter kola) is an economic and highly valued plant which is common in Western part of Africa. Virtually all the plant parts including the leaves have been widely used for various purposes [2]. The active components of the plant are flavanoids, xanthones and benzophenols [3]. The plant is used as antidote for poison or food contaminated by bacteria. The phenolic components of the plant possess anti-inflammatory, antimicrobial, antidiabetic and antiviral properties [4]. *Garcinia kola* has been widely researched by Africans due to its remedial potentialities and wide use in ameliorating various ailments. Most herbal products usually apply some quantity of *Garcinia kola* blended powder as additives to yield desired results. *G. kola* is believed to treat bacterial infections and viral infections [5]. *Zingiber officinale* (Ginger): This medicinal plant is commonly known as ginger; it is notable for its globally usage as spice. Its therapeutic application is due to its phytochemical constituents such as Alkaloids, Phenols, and high Oleoresin. Ginger is known to possess antibacterial, ameliorate inflammatory reactions and other effects (6). It contains gingerols as its primary bioactive compounds with high flavanoids, phytochemical

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and pharmacological effect [6]. Ginger is widely applied for the treatment of cold, poor appetite, viral infections, digestive disorders, arthritis, headache [7]. *Curcuma domestica* is known to have anti-inflammatory activity and inhibitory effect on carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth [8]. Cyclophosphamide is a chemotherapy drug that is used to treat cancer and autoimmune illnesses. It's utilized to quickly get the sickness under control. Because of its toxicity, it is quickly replaced by less toxic medications. To monitor kidney function, avoid drug-induced bladder problems, and screen for bone marrow toxicity, regular and frequent laboratory assessments are essential [9]. These three herbal medicines were parts of the major plants consume the most during this era of covid 19 pandemic. Some people believe it will protect them from contacting the virus while others think it will boost their immune system. The indiscriminate consumption of the plant by Nigerians necessitated this research. Hence, the aim of this research was to ascertain the Ameliorative Roles of *Zingiber officinale, Curcuma domestica, Garcinia kola* on the Cyclophosphamide effect on Histopathological biochemical Indices of Renal and Hepatic tissues of Albino Rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 *Equipment*

Automatic tissue processor, microtome, microtome knife, Spectrophotometer, microtome blade.

2.1.2 Biological Materials

Seeds of *Curcuma longa, Zingiber officinale, and Garcinia kola* were purchased in January 16th, 2020 and washed at New Benin Market, Benin city, Edo state. The plant materials were identified and authenticated by Mr. H. Akinbosun in the Department of Botany, University of Benin. The seeds were air-dried and ground into powder weighed and stored in an airtight container. A total of 40 rats (Wistar strain, male, 200 – 210g) were used in this study. The animals were obtained from the animal house of University of Benin and acclimatized for two weeks. They were fed with standard rat pellet (Grasscutter) and allowed free access to water.

2.2 Methods

2.2.1 Plant Extract

Five hundred grammes of combined extract of *Zingiber officinale, Curcuma longa, and Garcinia kola in ratio* 1:1:1, were macerated in 5000ml of 70% ethanol for four days with persistent stirring and shaking. Additionally, 150g powder extracts of each plant *(Zingiber officinale, Curcuma longa, and Garcinia kola*) were macerated in 1500 mL of ethanol separately for four days. The mixtures were filtered, and the extract concentrated to dryness on water bath at 50 °C. A Standard solution was prepared from the dried extract for oral administration.

2.2.2 Phytochemical Analysis

The phytochemical analysis of the extract was carried out using the method described by Owoyele et al., (2011), [10]

2.2.3 Ethical Consideration

The policies outlined in the Guide for the Care, Handling, and Use of Laboratory Animals were followed [11]. The ethical and promotion committee of the University of Nigeria Teaching Hospital, Etuku Ozuolla, Enugu, granted approval with approval number 056/02/2021.

2.2.4 Acute Toxicity Study

The acute toxicity study was done using a method described by Lorke [12]. The median lethal dose (LD_{50}) was calculated using the formula: Then the LD_{50} is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

2.2.5 Experimental Design Animal experimentation research design was used [13]. The rats were allowed to acclimatize for a period of two weeks at 25°C to 29°C The weight of the animals were taken and recorded at the start of the study. This experiment as carried out in two phases (1 and 2).
The first phase was divided into two groups, A and B and was fed for five-weeks.
Group A: Six rats (control group), received water and rat pellets. Group B: the rats in this group, which were randomly placed in five different cages of 7 rats in the first four cages and six rats in the last cage and when given 10 mg/kg body weight of cyclophosphonamide for five (5) weeks. Six (6) rats were randomly chosen from a total

of 34 rats after the fifth week, six rats were then removed from the five cages in group B. All of the rats in group A and the six (6) randomly chosen rats from group B were weighed before being anesthetized with chloroform inhalation. Blood samples were collected for the evaluation of renal and hepaticbiomarkers, and kidney and liver tissue were harvested for analysis. Group B was further divided into seven groups for the second phase of the work (n = 4 in each group). As a control, group A received only pelleted rat feed and water; group B received 600 mg/kg body weight of Zingiber officinale; group C received 600 mg/kg body weight of Curcuma longa extract; group D received only 600 mg/kg body weight of Garcinia kola extract; and group E received 600 mg/kg body weight of an equal proportion of combined extract (Zingiber officinale, Curcuma longa extract and Garcinia kola extract in ratio 1:1:1), Group F received 1200 mg/kg body weight of the mixture of the three plant extract in a ratio of 1:1:1, water and rat feed only, while group G received 2400 mg/kg body weight of the mixture of the plant extract in the proportion of 1:1:1, along with water and rat pellet only. All animals were weighed and anesthetized using chloroform, blood was drawn for a liver and kidney function test. Liver and kidney tissues were harvested for histological analysis.

2.2.6 Biochemical Analysis

Blood samples were collected for the estimation of Liver enzymes (AST and ALT, ALP) and two Kidney function indicators (Creatinin and Urea) using Randox diagnostic kits based on the principle described by Tietz *et al.*[13].

2.2.7 Histological Processing

Standard tissue processing procedure according to method described by Omorodion *et al,* 2020 [14] was followed

2. 2. 8 Staining Procedure

Standard staining technique described by Omorodion, *et al,* 2020 [14] was used

2.3 Statistical analysis

The data was analyzed using Analysis of Variance (ANOVA) and the results of the differences were considered significant at P< 0.05 level of confidence. All data were expressed as mean ± standard error of mean. The results were presented in tables and comparisons made statistically.

3. RESULTS

Photochemistry analytical findings

The plants' extracts were found to contain: tannin, terpenoids cardiac glycosides saponins, phlobatannins, cyanogenic glycosides, flavonoids, steroids, alkaloids and phenols.

Table 1: Effect of Cyclophosphamide on the Kidney and Liver Markers of treated rats against control

Parameters	Control	experimental	t-values	P-values
Urea	58.20±1.07	63.30±0.60*	-4.30	0.0001
Cr	0.90±0.01	1.27±0.02*	-8.06	0.0001
AST	7.10±0.10	8.60±0.14*	-6.08	0.0001
ALT	7.10±0.10	6.00±0.20*	-5.44	0.0001
ALP	40.10±0.06	79.60±0.80*	-23.31	0.0001

Keys: ALP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate aminotransferase), Cr (Creatinine)

Table 2: Effect of *Zingiber officinale, Cuecuma longa, Garcinia* kola and Combine Extract on liver and kidney markers pretreated with cyclophohamide

	Urea	Cr	AST	ALT	ALP
A	61.00±0.41	1.13±0.09	8.80±0.34	6.10±0.31	78.50±2.02
B	62.50±0.65	1.05±0.03	7.80±0.13*	5.20±0.06*	76.00±1.08
C	61.50±0.65	1.15±0.07	7.30±0.09*	5.30±0.06*	72.30±1.32*
D	61.30±1.32	1.08±0.05	7.10±0.04*	5.20±0.06*	75.50±0.65*
E	58.80±0.75	0.95±0.02*	7.10±0.13*	4.10±0.04*	43.00±0.58*
F	60.50±0.65	0.93±0.02*	7.00±0.09*	4.20±0.06*	45.50±0.65*
G	61.80±1.32	0.94±0.02*	7.30±0.05*	4.30±0.03*	46.00±0.91*

*Significant when compared with the control group*Significant when compared with the Group A

Keys: ALP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate aminotransferase), Cr (Creatinine)

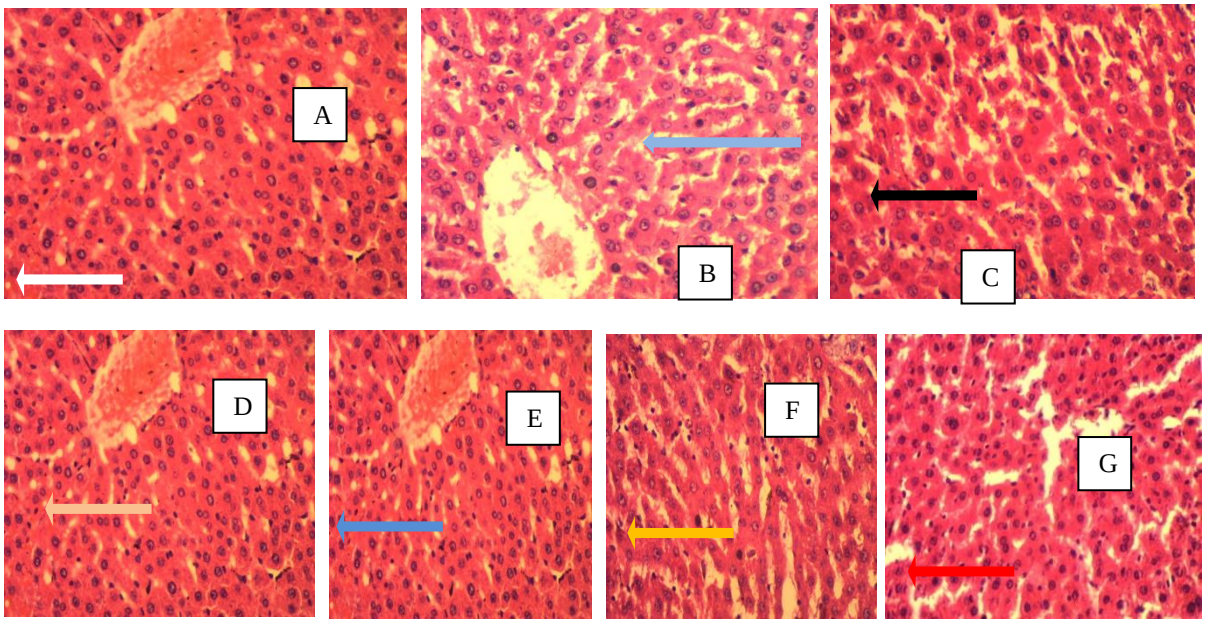

Fig 1: Section of rat liver treated with 10mg/kg of *cyclophosphamide*. Note the normal hepatocytes, section of rat liver treated with 600mg/kg *Zingiber officinale* extract. Note the normal hepatocytes with no necrotic changes (white and light arrow) (A and B). Section of rat liver treated with 600mg/kg *Curcuma domestica* extract. Note the normal hepatocytes (black and light purple) (C and D), section of rat liver treated with 600mg/kg *Garcinia kola* extract. Note the normal hepatocytes (blue arrow) (E), section of rat liver treated with 1200mg/kg pooled extract. Note the normal hepatocytes (purple)(F). Note the normal hepatocytes section of rat liver treated with 600mg/kg pooled extract. Note the normal hepatocyte (red arrow) (G), section of rat liver treated with 2400mg/kg pooled extract. H and E: X400.

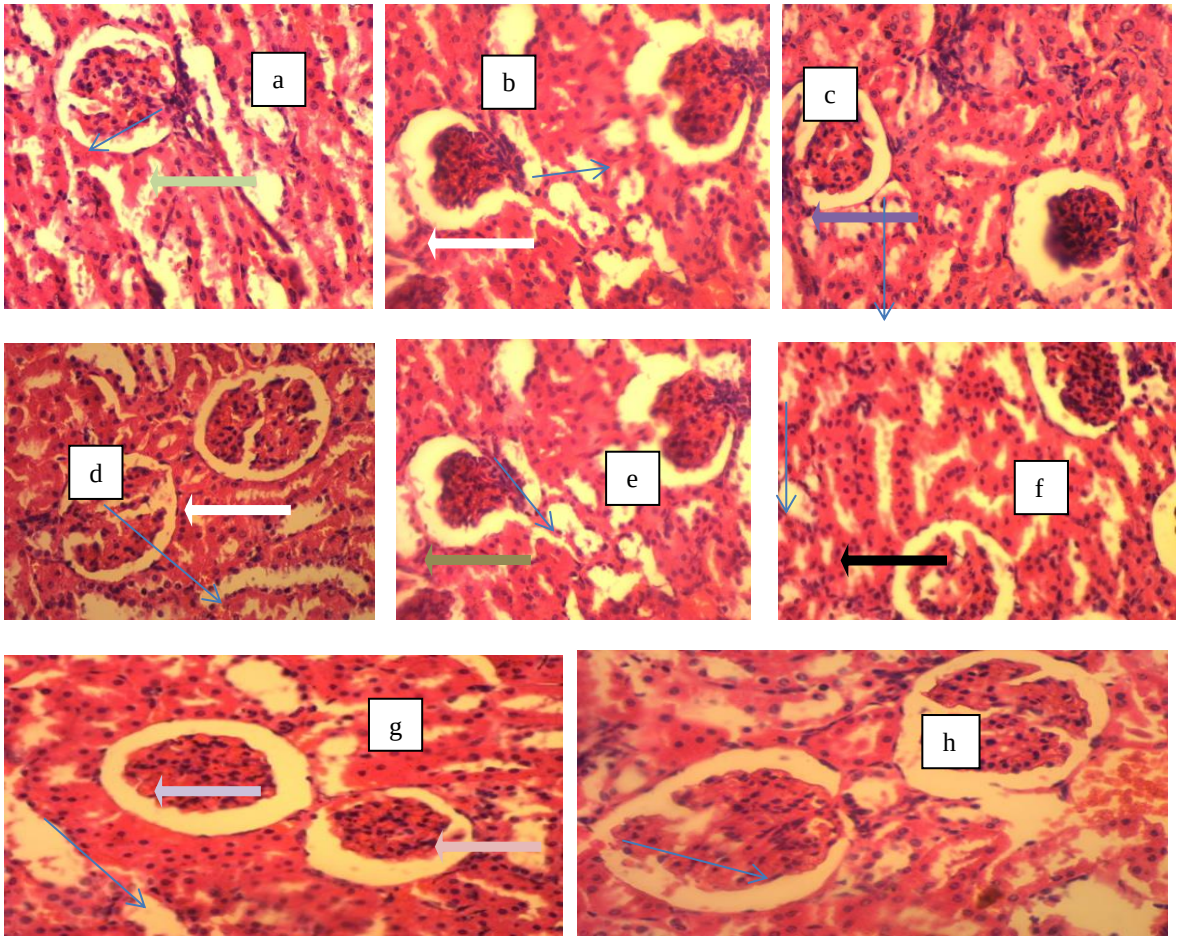

Fig 2: Section of the rat kidney (Control group). Note the normal glomerulus (olive green and white arrow) and normal tubules (thin arrow) (a and b), section of rat kidney treated with 10mg/kg cyclophosphamide. Note the erosion of the bowman capsules and mild tubular erosion (thin arrow) (c), section of rat kidney treated with 600mg/kg *zingiber officinale* extract (d and e). Note the normal glomerulus and normal tubules (purple and white arrow) , section of rat kidney treated with 600mg/kg *curcuma domestica* extract. Note the normal glomerulus (tan and black arrow) normal renal tubules (thin arrow) (f), section of rat kidney treated with 600mg/kg *garcinia kola* extract. Note the normal glomerulus (thick arrow)(g) and mild tubular eruption (thin arrow), section of rat kidney treated with 1200mg/kg pooled extract. Note the normal glomerulus (thick arrow) & normal tubules (light tan arrow), section of rat kidney treated with 2400mg/kg cooled extract. Note the normal glomerulus (tan arrow) and normal tubules (light red) (h). H and E X400mag.

4. DISCUSSION

Significant increase in urea, creatinine, ALP, AST and significant reduction in ALP was observed as shown in table 1. The ability of cyclophosphamide to either work on reducing adipose tissue or metabolize the fatty deposit may have contributed to the decrease in body weight. Dave Bridges conducted a similar study on the impact of cyclophosphamide and methotrexate on weight loss and some conclusions/recommendations regarding what may have led to weight loss were made [15]. Although increased adiposity is frequently linked to cancer survivorship, the underlying mechanisms are unknown. It is particularly challenging to separate the metabolic effects of the cancer from the therapy when using chemotherapeutic drugs. However, the widespread use of methotrexate for immunological conditions may provide some insights into how these medications affect energy balance apart from cancer and loss of weight, which in turn affects weight of the muscle [16]. In rats treated with cyclophosphamide, the value of creatinine increased significantly ($P < 0.05$), but the values of AST, ALT, and ALP decreased significantly (($P< 0.005$). Increased plasma levels of these enzymes by CP are a sign of cellular damage and a loss of the hepatocyte membrane's functional integrity, which causes their leakage into the serum or plasma· [16-17]. The liver's cytoplasm and mitochondria are rich in the enzyme AST, which is also found in the heart, skeletal muscle, and brain. The cytoplasm is where ALT, a hepato-specific enzyme, is primarily located [18–19]. Inhibition of intra- and extra-hepatic bile flow (cholestasis), hepatobiliary injury, and overproduction or leakage of ALP and GGT are all signs of cholestasis, according to research on ALP and GGT, which are linked to cell membranes [20, 21]. The result in table 2 showed the levels of AST and ALT were significantly lower in groups B through G than the control, while the levels of ALP were significantly higher in groups C through G and the levels of creatinine were significantly higher in groups E - G. Following administration of various concentrations of the herbal preparation, there were no statistically significant changes in the values of creatinine and urea. The active ingredients in the extract of ginger, turmeric, and bitter kola may be responsible for the decline in some of the markers. Tannin, terpenoids, cardiac glycosides, saponins, phlobatannins, cyanogenic glycosides, flavonoids, steroids, alkaloids, and phenols are a few of these active ingredients [22]. These phytonutrients have a variety of uses. Due to their antioxidant properties, flavonoids are known to play a protective role in humans [23]. Alkaloids are widely used as stimulants, narcotics, anti-malarials, anti-protozoans, and anti-bacterials [24]. They are also known to have protective effects against herbivores and pathogens. Due to the phenol content of tannin, which is known to have antiseptic properties, it is also used in the treatment of many infectious diseases [25]. By increasing myocardial contraction, cardiac glycosides have been shown to be effective in the treatment of congestive heart failure [26]. The photomicrograph reveals that there was mild glomerular erosion, which may have affected the nephron of the kidney's ability to selectively reabsorb essential nutrients during filtration. Following administration of the plant extracts across all test groups in increasing doses (600 mg/kg, 1200 mg/kg, 2400 mg/kg of *Zingiber officinale*, *Curcuma domestica*, *Garcinia kola*, combined extract, this slight tissue alteration was restored. No noticeable changes were seen in the animals' livers before or after administration, probably as a result of the liver's detoxifying properties. Some researchers claim that there are no negative effects of CP on the kidney. This is due to the fact that its by-product, acrolein, differs from our findings in that it is more urotoxic than nephrotoxic [26]. However, in some other studies, which have confirmed pathological alterations such as acute inflammatory cell infiltration into the cortex, tubular cell oedema, brush border loss, and ultimately pyknotic cells [27]. Sakr et al [27] who administered cyclophosphamide and noted dilated, congested renal blood vessels, vacuolations of epithelial lining renal tubules, and atrophy of glomerular tuft also suggested similar changes. According to the increase in oxidative markers in the kidney tissues, the changes were attributed to cyclophosphamide-induced oxidative stress [27]. This subsequent discovery was consistent with the results of our study. These degenerative potentials may change the nephron's capacity for filtering and selective reabsorption. *Curcuma longa*, *Zingiber officinale and Garcinia kola* combined therapeutic properties on the above degenerative changes were largely attributed to their abundant phytonutrient content [22].

5. CONCLUSION

Our present studies have shown that ginger, turmeric and bitter kola can improve kidney and liver function individually and collectively. This could be a cheaper means of treatment if taken in prescribed dosage and could also serve as food supplement in enhancing nourishment.

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Conflict of Interest
The authors declare no conflict of interest

Contribution of the Author
The research title was coined and conceptualized by Godfrey I. Iyare, the introduction was drafted by Nosa T Omorodion, practical aspect conducted by both authors, the results and discussion computed and drafted by both authors.

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