

EFFECT OF COMBINED AQUEOUS LEAF EXTRACT OF *Mentha spicata* AND *Murray Koenigii* ON ACETAMINOPHEN (paracetamol) Induced Hepatic Damage on Wistar Rats

Abstract

The study investigated the effect of combined leaves extract of *Mentha spicata* and *murray koenigii* leaves on acetaminophen induced hepatic damage on wistar rat. Twenty five (25) wistar rats with weights range of 100g-130g were used for this study. They were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were kept in the animal house of the University. They had free access to water and feed during the period of the experiment. Treatment of test animal with 80mg/kg, 120mg/kg, 150mg/kg dose of the leaf extract showed ameliorative effect on the animals with specified dose producing the best result. The significant increase in liver marker enzymes (AST, ALP, and AST) activities of acetaminophen-induced rats caused by liver injuries which resulted in leakage of liver enzymes to the extra hepatic tissue due to compromised liver architecture and permeability. The improvement of the effect of the acetaminophen suggests that these combined leaves action can be of valuable use in the management of hepatic injury caused by this toxic action.

INTRODUCTION

Many antioxidants prevent the lipid peroxidation of free radical effect on cells and tissues. The herbs and spices play conspicuous role in human nutrition and therefore can be termed as nutraceuticals. Herbs and spices are important in human life as they are good source of different antioxidant compounds, which have therapeutic effects against cancer and cardiovascular diseases. Peppermint (*Mentha piperita* L.), belonging to the Labiatae family, is a large family of annual or perennial, herbaceous plants of 30 – 100cm height, which is cultivated in temperate climates, in America, Europe and Asia (Arslan *et al.*, 2010). Peppermint (*Mentha piperita* L.) is a natural hybrid of water mint (*Mentha aquatic* L.) and spearmint (*Mentha spicata* L.). It is cultivated globally for its use as a flavoring in foods and in some shampoos and soap (Tarham *et al.*, 2010). Mint is one of the most important and common flavors in the world coming after vanilla and citrus flavours (Arslan *et al.*, 2020).

Peppermint oil and some of its constituent is known for antimicrobial and antioxidant properties and it is one of the most widely consumed single ingredients in herbal teas. The essential oil of peppermint is used in traditional medicine (Lv *et al.*, 2012). Peppermint has significant antimicrobial and antiviral activities, and also possesses strong antioxidant and antitumor actions and exhibits some antiallergenic potentials (Skalicka-Wazniak & Walasek, 2014). A variety of volatile compounds, mainly menthol, menthone and isomenthone have been identified along with β -carotene, chlorophyll, δ - and γ -gamma tocopherols and ascorbic acid (Figuerola Perez *et al.*, 2014). Plant pigment is a generic

expression used to designate a large number of coloured molecules. Based on their chemical structure, they can be classified into 5 families, namely; i.e tetrapyrroles (e.g chlorophyll), carotenoids (e.g β -carotene), phenolic compounds, e.g. teaflavin and N-heterocyclic compounds (e.g betalains) (Schoefs, 2002).

Appreciable amount of carotenoids are present in fresh tea leaves, but this value is greatly decreased during tea processing, leading to various degradation products (Ravichandra, 2002). In a Japanese study on green teas 38 different pigments were detected (6 of them were unknown), (Suzuki & Shioi, 2003). Among these tea pigments, pheophytins a and b were abundant, followed by chlorophylls a and b, and carotenoids such as β -carotene and lutein in lower concentration. All these pigments exhibited significant antioxidant activities against hydrogen peroxide generation, in the order chlorophyll a > lutein > pheophytin a > chlorophyll b > β -carotene > β -carotene > pheophytin b (Lorant et al., 2010).

Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process (Ong & Tee, 1992) and also protect chlorophyll from photooxidative destruction (Sieferman-thams, 1987; Giri et al., 2013). Scanty reference exist on the elemental constituents of mint and curry leaves (Zienal et al., 2003). The objective of this study was to evaluate the combined effect of curry and mint leaves on acetaminophen toxicity of wistar albino rat, and to determine the hepatotoxicity of Acetaminophen (paracetamol).

MATERIAL AND METHOD: Fresh leaves of mint (*Mentha spicata*) and curry (*Murraya Keonigii*) were collected from Woji in Obio-Akpor local government area of Rivers state and identified in the herbarium of the department of plant science and biotechnology in the university of Port-Harcourt. The leaves were washed and air dried in the room temperature. The leaves were ground into fine powders to increase their surface area. 2.5g of mint leaf and 2.5g of curry leaf were dissolved in 50ml distilled water and shaken vigorously for five minutes, allowed to stand for 10 minutes and then shaken for another five minutes and allowed to stand for 24 hours. This was filtered first with a piece of white cotton cloth for five times and then with the Whateman filter paper. The filtrate served as stock from which dilutions of 80mg/kg, 120mg/kg and 150mg/kg were prepared and used for the treatment.

Experimental Animals

Twenty five wistar rats with weights of 100-130g used for this study were obtained from the animal house of the department of Biochemistry, University of Port Harcourt, Nigeria. They were also kept in the animal house where they had free access to clean drinking water and feed. Variable factors such as light temperature and humidity were also put into account and maintained throughout the experiment.

Experimental Design

The 25 albino rats were randomly divided into five groups. Group 1 served as normal control and received only feed and water. Group 2 served as the negative control which was induced with paracetamol orally but was not treated with extract. Group 3-5 were induced with acetaminophen and treated with different concentrations of the extract. This lasted for seven days.

Collection of Blood Sample

The group 1-5 animals were sacrificed after 7 days. They were anaesthetized in chloroform saturated chamber, and sacrificed. Fresh blood was extracted and placed in lithium heparin bottles. Liver samples were collected into sterile bottles for analysis.

Biochemical Assays

The determination of aspartate aminotransferase (AST) in the sample were performed at 37°C using the Colorimetric method using the randox kit by measuring the amount of oxaloacetate hydrazone formed in the presence of L-aspartate, D-oxoglutarate and 2,4-dinitrophenyl hydrazine as reported by (Ibekwe *et al.*, 2007). For alanine amino transferase (ALT), L-alanine replaced L-aspartate using the Colorimetric method. The determining of alkaline phosphatase activity used the diagnosticum kit and monitored the amount of inorganic phosphate releases from p-nitro Phenyl phosphate following the procedure of Thaussant (1977). The colorimetric method was used to determine triglycerides (TRIGS) and cholesterol (total cholesterol) and HDL cholesterol.

Statistical analysis

All data were expressed as mean $\pm$ SEM and statistically analyzed with the analysis of variance (ANOVA) at 95% confidence level. A P value of < 0.05 was considered statistically significant.

RUSILTS

Tables 1-6 below show the result. The value of ALT, cholesterol, Triglycerol, high Density lipoproteins (HDL), LDL and ALP showed increase in all the parameters above (122-30mmol/l). There was an uneven increase in Triglyceride, and the same was observed for HDL, LDL, ALP.

Table 1: Alanine Aminotranferase

Table 2: Cholesterol

Table 3: Tricylglycerol

Table 4: High Density Lipoproteins

Table 5: Low Density Lipoproteins

Table 6: Alkaline Phosphates



Table 1: Alanine Aminotranferase

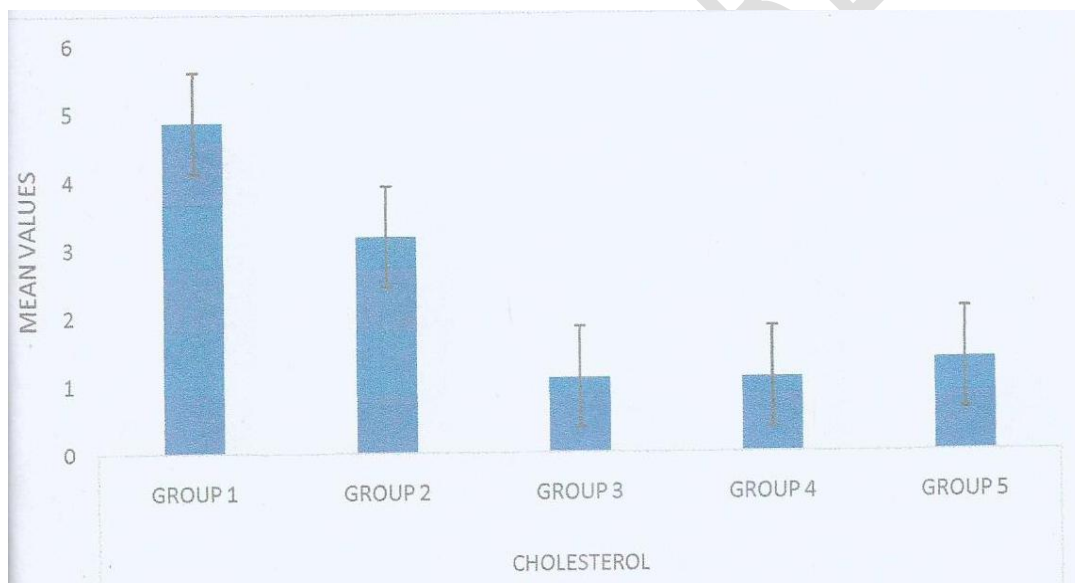


Table 2: Cholesterol

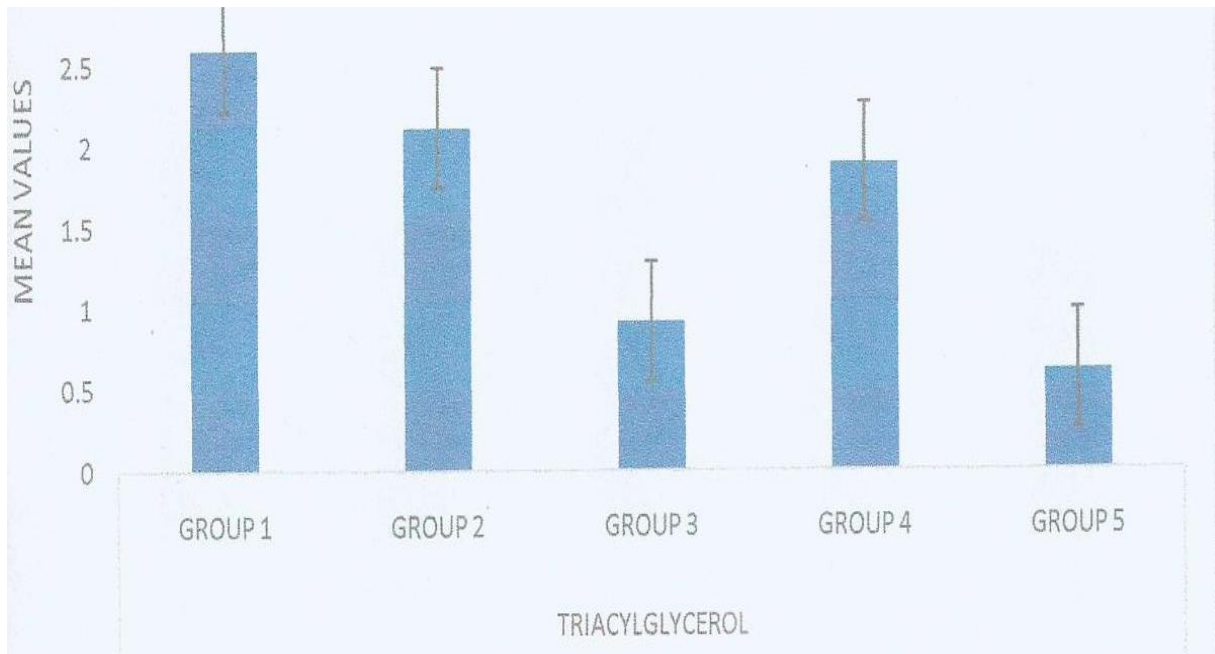


Table 3: Tricylglycerol

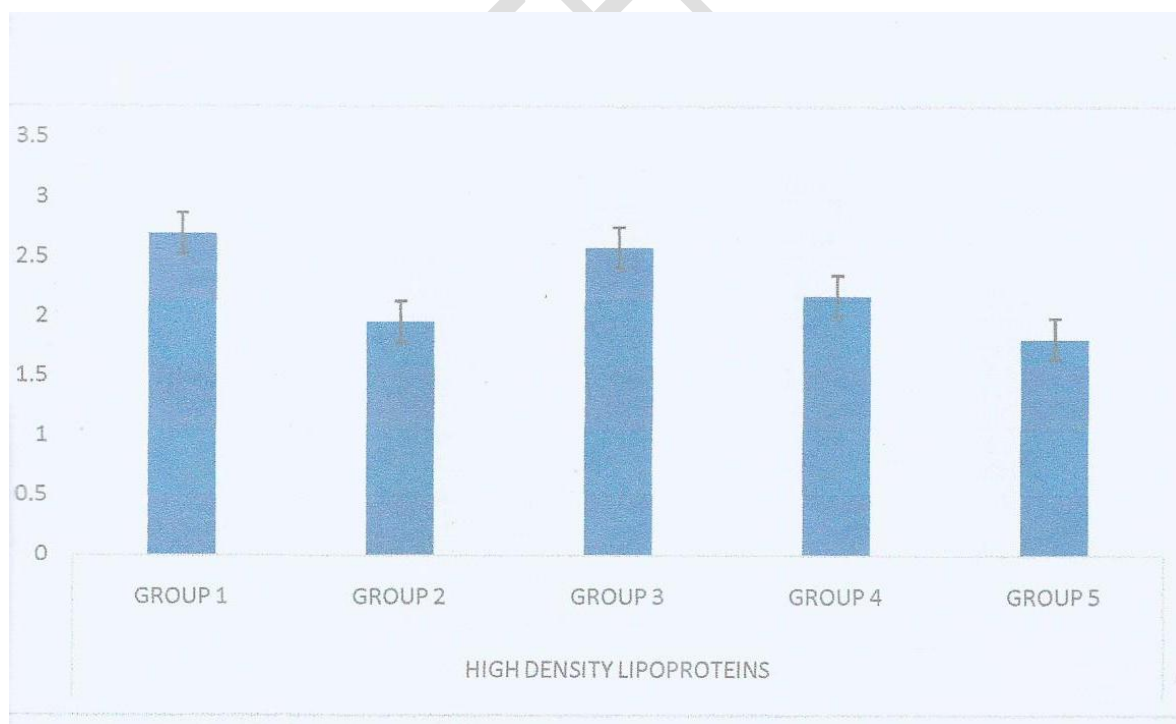


Table 4: High Density Lipoproteins

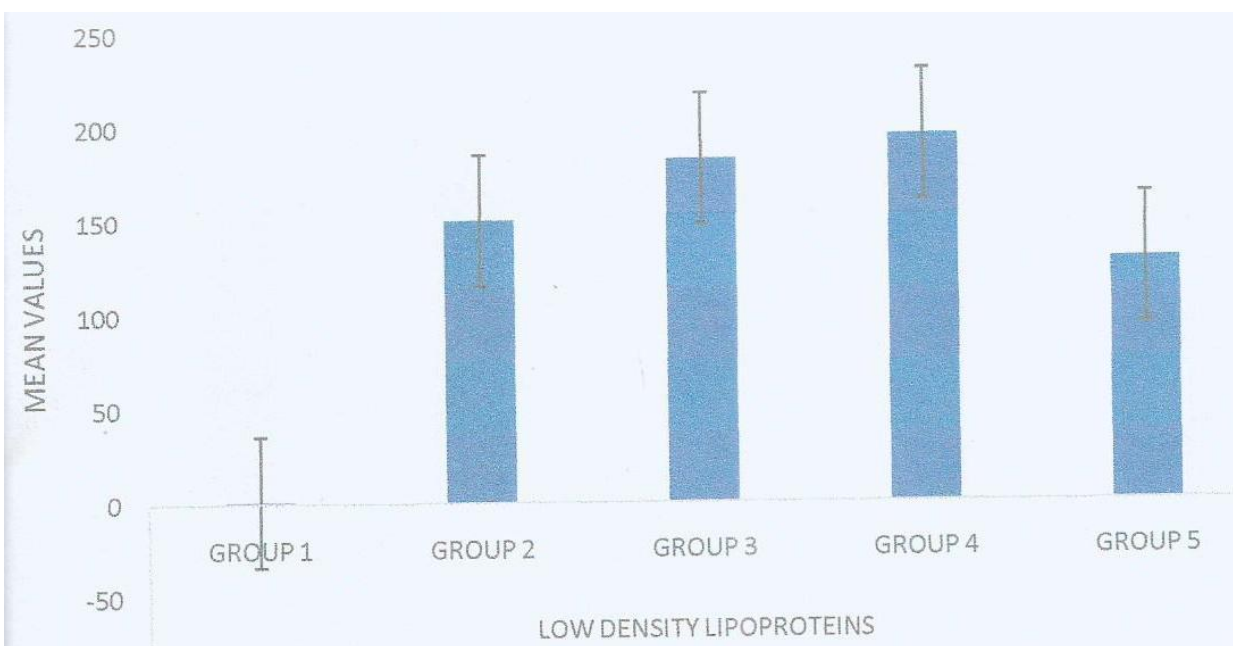


Table 5: Low Density Lipoproteins

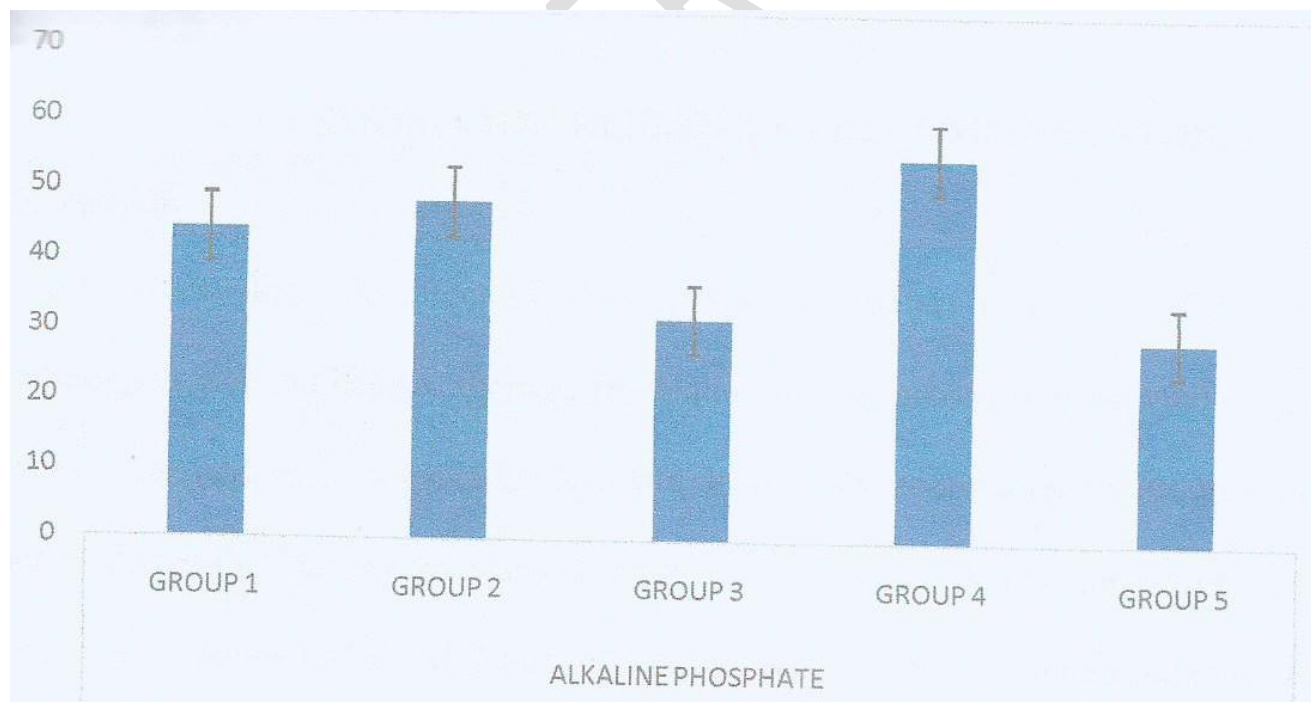


Table 6: Alkaline Phosphatase

Discussion

The significant increase in liver marker enzymes (AST, ALP and AST) activities of acetaminophen-induced rats was caused by the leakage of liver enzymes to the extrahepatic tissue. The increased liver marker enzymes activities could be attributed to hepatic failures such as acute hepatic necrosis and destruction of hepatic cell membrane that compromise liver integrity and permeability (Haussament, 1977).

The hepatic failure could have occurred from lipid peroxidation by reactive N-acetyl-p-benzoquinone-1-imine, which is produced from acetaminophen breakdown by cytochrome P450 enzymes. There was an increase in AST in the treated group when compared to negative group. The extract contained bioactive constituent that stimulated the continuous leakage of ALT (Ibekwe et al., 2007).

The glucose concentration showed that there was a decrease in treated groups compared to group 2 showing hypoglycemic activity. The ALT concentration decreased in the groups 3 and 5 compared to group 2. Total cholesterol concentration decreased significantly ($p < 0.05$) in the treated group compared to group 2. Triacylglycerol (TAG) Significantly decreased ($p < 0.05$), in groups 3 and 5 when compared to group 2. There was no significant decrease in group 4 rats. Good cholesterol, the resultHDL, showed positive result for group 5 rats ($p < 0.05$). The result suggested that the extract has antilipidemic activity. There was also a decrease in LDL for group 5 rats (Ibekwe *et al.*, 2007).

Conclusion

In conclusion, the study showed that the combined action of the extracts posses hepatoprotective effect on acetaminophen – induced wistar rats.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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