

Full length Research Article

## Antihyperlipidemic Property of *Murraya koenigii* Ethanolic Leaf Extract on Rats Fed with Cholesterol-Enriched Diets

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**Summary:** Hyperlipidaemia, a family of disorders characterized by abnormally high levels of lipids in the blood is associated with increase in the risk of coronary heart diseases. Medicinal plants contain numerous biologically active compounds which are helpful in the treatment of various diseases and improving human life. *Murraya Koenigii* (MK) has been reported to possess important phytochemicals and has been employed in the treatment of a wide range of diseases. This study was therefore, designed to investigate the antilipidaemic property of ethanolic leaf extract of *Murraya koenigii* on rats fed with cholesterol-enriched diets for 14 days. The study was carried out on 20 male wistar rats divided into 4 groups (n=5). Group A was the control group and received normal rat chow, group B received cholesterol-enriched diet (CED); while groups C and D received cholesterol-enriched diet and were treated with 200 mg and 400 mg/kg body weight of ethanolic extract of *Murraya koenigii* leaf respectively. Blood samples were collected by cardiac puncture on the last day of the experiment for biochemical analysis. Results showed a significant increase ( $p \leq 0.05$ ) in Total cholesterol (TC) level ( $117.4 \pm 4.6$ ) and low density lipoprotein cholesterol (LDL-c) level ( $50.1 \pm 4.7$ ) of group B rats when compared to other groups. The MK (400 mg/kg) treated rats, showed a significant reduction in TC and LDL-c levels, and a significant increase in HDL-c level when compared to CED group. However, there were no significant changes in all the lipid parameters of the treated groups when compared to the control. This study showed that cholesterol-enriched diet caused dyslipidaemia and *Murraya koenigii* exhibited significant hypolipidaemic effect on serum TC and LDL-c and increased HDL-c. Hence, *Murraya koenigii* exhibited antihyperlipidaemic properties.

**Keywords:** *Murraya koenigii*, hyperlipidaemia, Lipid profile, Cholesterol-enriched diet, Wistar rats

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### INTRODUCTION

Hyperlipidaemia is a family of disorders characterized by abnormally high levels of lipids (fats) in the blood. Although fats play a vital role in the body's metabolic process, high blood levels of fats increase the risk of coronary heart diseases (Beresford *et al.*, 2006; Howard *et al.*, 2006; Mallick and Khan, 2016). Dyslipidaemia has been considered as one of coronary risk factors contributing to the cardiovascular diseases (Rohan *et al.*, 2019). Dyslipidaemia is a condition in which the lipid levels are altered in the body and characterized by abnormal lipid status such as triglycerides, cholesterol and phospholipids in the blood. It comprises of elevated blood concentrations of low density lipoprotein cholesterol (LDL-c), triglycerides and decreased high density lipoprotein cholesterol (HDL-c) (Musunuru, 2010; Vallamkondu and Ajay, 2022).

Medicinal plants contain numerous biologically active compounds which are helpful in the treatment of various diseases and improving human life. In addition to being a good source of anti-infective agents, they are also cost effective and have fewer side effects (Samson *et al.*, 1991; Nayak, 2006). *Murraya koenigii* is commonly known as curry leaf plant and belongs to the family *Rutaceae*. It is a native of India, Sri Lanka and other South Asian countries (Singh *et al.*, 2011; Kumar *et al.*, 2015), and cultivated in

other parts of Africa, including Nigeria. It is used as a spice due to their distinctive flavor and aroma. The major chemical constituents of this plant as reported are carbazole, alkaloids, coumarins, flavonoids (Kumar *et al.*, 2015; Molly *et al.*, 2017). Traditionally, the leaves of this plant are used in cooking as well as treatment of a wide range of diseases and disorders such as pain, inflammation, itching, cancer, diabetes and blood disorders (Singh *et al.*, 2011; Kumar *et al.*, 2015). The increasing use of plants to treat and prevent various diseases has raised a concern especially regarding lipid profile as dyslipidaemia is considered a major risk factor for cardiovascular diseases. Hence, there is a need to research our traditional plant extracts in overcoming the hyperlipidaemic effects associated with consumption of high fat diets.

The beneficial effect of *Murraya koenigii* leaves on cholesterol induced hyperlipidaemia in rats is sparsely reported in the recent literature. Therefore, the present study was aimed at investigating the antilipidaemic property of ethanolic leaf extract of *Murraya koenigii* on rats fed with cholesterol-enriched diets

### MATERIALS AND METHODS

**Plant Collection and Identification:** Fresh leaves of *Murraya koenigii* leaves were collected from its natural

habitat in Abakaliki, Ebonyi State. Identification and authentication was done at the Botany department of the Ebonyi State University, Abakaliki. The leaves were washed and air dried at room temperature for 3 weeks

**Plant Material and Extraction:** Extraction was done by modification of method of Kesari et al., (2005). *Murraya koenigii* ethanolic leaf extract was prepared by cold maceration. Five thousand grammes (5000 g) of powder was soaked in 3 litres of ethanol for 7 days, the extract was decanted and the remaining filtered off using Whatman filtered paper. The extracted analytes were concentrated by distilling off the solvent and evaporated to dryness on a water bath at 40°C to obtain a pasty dark-green extract with a characteristic aromatic smell. The extract was then weighed and kept safe in the refrigerator.

**Experimental Animal:** A total number of twenty (20) male wistar rats weighing between 150-200 g used for the study were purchased from animal house of the Physiology Department, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State. The rats were acclimatized for 7 days prior to the study and fed with normal rat chow. The animals were kept in cages and maintained under standard conditions with light-dark cycle and were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals. The Departmental Ethical Committee granted approval for the conduct of the research following research proposal presentation.

**Formulation of Cholesterol Enriched Diet:** The cholesterol enriched diet was prepared following the modifications of the recommendations of the American Institute of Nutrition (AIN, 1977). The diet consisted of 60% fats and 40% of other constituents. The 60% fats consist of animal lard, butter and egg yolk. The 40% of other constituents consists of powdered milk, sucrose, soya beans, groundnut, rice husk, and yellow corn. The animal lard was heated and the oil extracted, while the yellow corn, soyabeans, groundnut were selected and ground separately using the local grinding machine. Then, the eggs were broken and the yolk extracted. A measured quantity of 126 g each of the ground soyabeans, groundnut, yellow corn, powdered milk, sucrose, rice husk, were measured into a bowel respectively. Also, 378 g each of butter, animal lard and egg yolk were measured and put into the bowel. All the measured constituents were mixed together and little water was added. Thereafter, they were molded into balls weighing about 90 g and oven baked at a temperature of 40°C for 30 minutes. The balls were left to cool and solidify. After which, they were stored in the refrigerator from where they were taken from to feed the rats.

**Experimental Design:** The animals were randomly selected into four groups of five rats per group (n=5)  
 Group A: Normal control received normal rat chow  
 Group B: Experimental control received cholesterol-enriched diet.  
 Group C: Received cholesterol enriched diet and 200 mg/kg body weight of *Murraya koenigii* extract.  
 Group D: Received cholesterol enriched diet and 400 mg/kg body weight of *Murraya koenigii* extract.

All groups were given access to drinking water (distilled water) ad libitum.

**Sample Collection and Analysis:** Body weights of the animals (before and after administration) were measured. All administrations were done orally using oropharyngeal cannula once per day for 14 days. On the last day of experimental study, all the animals were sacrificed. Blood samples were collected by cardiac puncture into plain tubes, centrifuged at 3000rpm for 10 minutes and the serum was separated and stored frozen until the time of biochemical analysis.

**Biochemical Assay:** HDL and TC were measured by colorimetric method using commercial diagnostic kit (Randox) and TG (by Tecno Diagnostics) according to the procedure described in the manufacturer's operation manual. VLDL and LDL were estimated using Friedewald's equation (Friedewald et al., 1972) as:  
 $VLDL = TG (mg/dl)/5$ ;  $LDL = TC - (HDL + VLDL)$

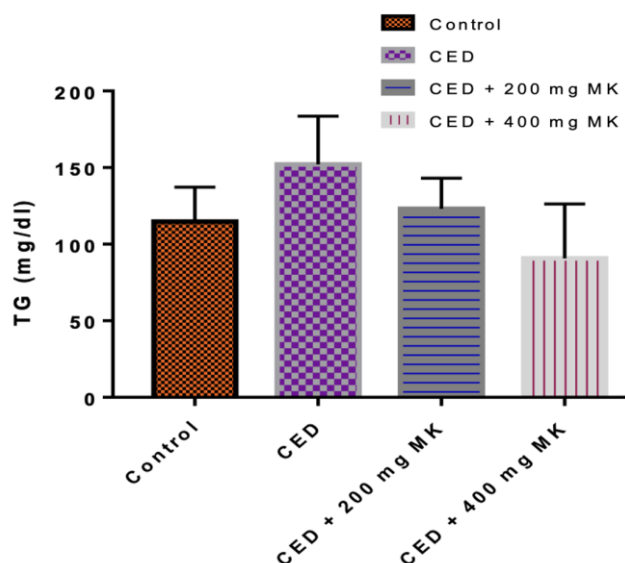
### Statistical Analysis

Data were expressed as mean  $\pm$  SEM. Data were analyzed using Graph pad prism 7.0 software. Differences in means were compared using ANOVA and Students t-test. P-values  $\leq 0.05$  were taken as statistically significant.

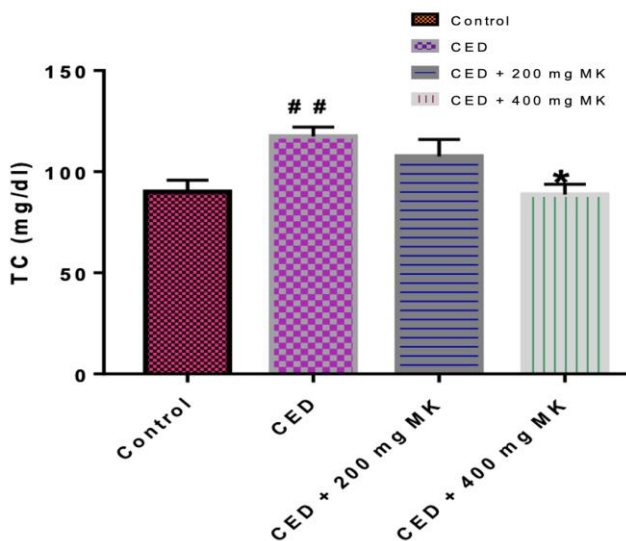
## RESULTS

**Effect of Ethanolic Extract of *Murraya koenigii* (MK) Leaf on Lipid Parameters of Rats Fed with Cholesterol-Enriched Diets (CED):** The effect of *Murraya koenigii* on Triglycerides (TG), Total Cholesterol (TC), High Density Lipoprotein cholesterol (HDL-c), Very Low Density Lipoprotein cholesterol (VLDL-c) and Low Density Lipoprotein cholesterol (LDL-c) are shown in figures 1-5 below.

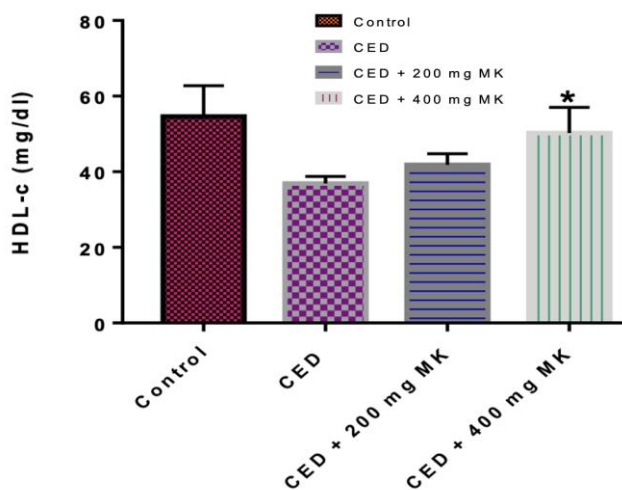
Oral administration of *Murraya koenigii* leaf extract has no significant effect ( $p < 0.05$ ) on the TG and VLDL-c levels of the rats fed on Cholesterol-Enriched Diets as shown in figures 1 and 4.



**Figure 1:** Effect of *Murraya koenigii* leaf extract on triglycerides (n=5)



**Figure 2:** Effect of *Murraya koenigii* leaf extract on Total Cholesterol (n=5; ##P<0.01 vs control group; \*p<0.05vs CED group)



**Figure. 3:** Effect of *Murraya koenigii* leaf extract on High Density Lipoprotein-cholesterol (n=5; \*p<0.05vs CED group)

Figure 2 shows the effect of *Murraya Koenigii* leaf extract on TC level. There was a significant increase in the TC level of the rats fed on Cholesterol Enriched Diets (CED) when compared to control group. In the MK (400 mg/kg) treated rats, significant reduction in the TC level was observed when compared to the CED group.

As indicated in Figure 3, there was a significant increase in the HDL-c level of rats that received MK (400 mg/kg) when compared to the CED group. Figure 5 shows the effect of *Murraya koenigii* on LDL-c. In group B (CED), there was a significant increase ( $P \leq 0.05$ ) in LDL-c levels when compared to the control while in the MK (400 mg/kg) treated rats, there was significant reduction in the LDL-c level when compared to the CED group

## DISCUSSION

Cholesterol-enriched diet/high fat diet intake is known to increase saturated and trans fatty acids which may be associated with hyperlipidaemia and also increases body

weight through several mechanisms. This is because it contains a greater percent of fat than other constituents. Also, lifestyle changes such as lack of physical exercise, high consumption of fat-enriched diet give rise to metabolic syndrome (Rivera-Ramirez *et al.*, 2011). This metabolic syndrome may include hypertension, insulin resistance, dyslipidaemia and cardiovascular disease.

The increase in total cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) levels seen in rats fed with cholesterol-enriched diet (CED) is consistent with the report of Rohan *et al.*, (2019), who reported an increase in serum lipid parameters mainly total cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) when rats were fed with high fat-fructose diets.

Meanwhile, the increased HDL-c, and decreased TC and LDL-c observed in the MK treated group is in agreement with the report of Ekoh *et al.*, (2014) who reported a decrease in TC, LDL-c, VLDL-c, and TG and an increase in the level of HDL when *Murraya koenigii* was used together with *Ocimum gratissimum* and *Piper guineense* in diabetic rats. It is also consistent with the report of Rohan *et al.*, (2019), who reported that *Murraya koenigii* leaves extract exhibited hypolipidaemic effect on serum TC and LDL-c of High fat-fructose fed rats. However, it is not in agreement with the report of Lawal *et al.*, (2008) who reported a decrease in TG level and an insignificant effect on TC, HDL-c, LDL-c levels in diabetic rats when treated with *Murraya koenigii* leaf.

The observed increase in TC and LDL-c levels of the rats that received cholesterol-enriched diet may be attributable to the dyslipidaemic effect of the diet. Dyslipidaemia is a condition characterized by abnormal lipid status such as triglycerides, cholesterol and phospholipids in the blood. It comprises of elevated blood concentrations of low density lipoprotein cholesterol (LDL-C), triglycerides and decreased high density lipoprotein cholesterol (HDL-c) (Mooradian, 2009; Musunuru, 2010; Kopin and Lowenstein, 2017).

The phytochemical properties of *Murraya koenigii* have been reported for various pharmacological activities (Rivera-Ramirez *et al.*, 2011; Kumar *et al.*, 2015 and Molly *et al.*, 2017). According to Thomas *et al.*, (2017) *Murraya koenigii* leaves increase HDL level by activating an enzyme HDL-associated paraoxanase and also protects this enzyme from cell damage. There is also a redistribution of lipoprotein due to the presence of bioactive compounds like flavonoids and phenolics (Patel *et al.*, 2013). So the bioactive compounds are involved in depleting TC levels through two possible mechanisms: reducing fat absorption in the digestive system and increasing fat excretion into faeces (Thomas *et al.*, 2017). Ijaz *et al.*, (2022) also proposed a plausible reason for the mechanism of lowering LDL and cholesterol levels; that curry leaves inhibit absorption of dietary cholesterol. It is also possible that curry leaf might mobilize extrahepatic cholesterol to the liver where its breakdown and final elimination takes place. In conclusion, results from this study suggest that *Murraya koenigii* leaf has antihyperlipidaemic properties which might be due to its presence of bioactive constituents

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