

THERAPEUTIC ROLE OF CURRY LEAF (*MURRAYA KOENIGII*) EXTRACTS ON LIPID METABOLISM IN WISTAR RATS

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Abstract

The aim of this research project is to evaluate the effect of Murayya koenigii leaves on dyslipidemia Wistar rat. Thirty Six (36) male rats were randomly divided into six groups of six (6) animals each. High Fat Fructose Diet (HFFD) composed of high fructose (10% fructose) added in drinking water bottle and high fat (butter (vanaspati ghee): coconut oil in ratio of 3:1) mixed with an egg yolk in dough of animal food diet. was fed per oral (p.o) to all rats from Groups I, II, IV, V and VI except Group III throughout the period of 14 weeks. Group III rats received normal diet and water ad libitum only. Group I, II, IV and V were treated respectively with Aqueous Extract of Murayya koenigii Leave (AEMK) (200 mg/kg/day, p. o), Methanolic Extract of Murayya koenigii Leave (MEMK) (200 mg/kg/day, p. o), Metformin (MET) (50 mg/kg/day, p. o) and Atorvastatin (ATO) (10 mg/kg/day, p. o). On the last day of experimental study, blood was collected by retro-orbital puncture method. Blood Sugar Level (BSL) and lipid profile were assessed. Results showed elevated levels of Total Cholesterol (TC), Triglycerides, Low Density Lipoprotein-Cholesterol (LDL-C), Very Low Density Lipoprotein-Cholesterol (VLDL-C) and diminished level of High Density Lipoprotein-Cholesterol (HDL-C) were observed in group VI. Murayya koenigii leaves extract exhibited significant hypolipidemic effect on serum Total Cholesterol (TC) and Low Density Lipoprotein-Cholesterol (LDL-C) in rats owing to its hypocholesterolemic properties. Atherogenic Index of Plasma (AIP) was highly significant in both of Aqueous Extract of Murayya koenigii Leave (AEMK) and Methanolic Extract of Murayya koenigii Leave (MEMK) extracts. Results of the present study have suggested that the antihyperlipidemic activity of Murayya koenigii leaves leading to decrease in serum lipid parameters mainly Total Cholesterol (TC), Low Density Lipoprotein-Cholesterol (LDL-C) along with atherogenic risk might be due to its presence of bioactive compounds.

Keywords: *Murraya koenigii, wistar rat, dyslipidemia, lipid parameters.*

INTRODUCTION

Dyslipidemia is defined as an abnormality in or an abnormal amount of serum lipids or lipoproteins in the blood (Azahan, Mani, Ramasamy, Lim, James & Alsharidah, 2020). As per NCEP-ATP III Guidelines Serum Cholesterol >200mg/dl, LDL-C >100mg/dl, Triglycerides >150mg/dl and HDL-C <40mg/dl are characterized as dyslipidemia. Dyslipidemia is an independent and modifiable risk factor of cardiovascular disease (Franyoto, Nurrochmad & Fakhrudin, 2024). To combat dyslipidemias, patients have several treatment options that include drugs, diet, and lifestyle changes. Drugs are effective but produce adverse effects in a significant proportion of patients.

Statins are the most widely prescribed lipid-lowering drugs worldwide, but not all patients respond sufficiently to this. They produce adverse effects in up to 33% of patients (Ahmed, Sunil, Cheekavolu & Alasyam. 2017). On the other hand, therapies based on dietary and lifestyle changes produce little or no adverse events and are the cornerstone of recommendations by the US national cholesterol education

programme (He, 2022). Dyslipidemia is the most important atherosclerotic risk factor (Vengala, 2017). In Nigeria, the disease is a significant health concern with increasing rates due to changes in lifestyle and diet. Studies have shown a high prevalence of dyslipidemia in both urban and rural population across different age groups. Recent studies have reported that high cholesterol is present in almost 25 to 30% of urban and 15 to 20% of rural subjects (Patterson & Verghese, 2015). Another review highlighted the association between dyslipidemia and other chronic conditions such as hypertension, diabetes and obesity in the Nigerian population (Tembhurne & Sakarkar, 2012). These co morbidities further increase the risk of developing cardiovascular disease. In a country like India the most common dyslipidemias prevalent are borderline high LDL cholesterol, low HDL cholesterol and high triglycerides (Thiyagarajan & Kanchana, 2023). Focus on dyslipidemia management is urgently required in India to hold the rising tide of coronary heart disease (Phatak, Khanwelkar, Matule, Datkhile, Hendre, 2019). Since human's existence on planet, man has been dependent on nature for curing various diseases. Natural plants and herbs are a rich source of lead compounds many of which are useful drugs in themselves (Acimovic, Jaromela, L. Pezo, Kiproviski, & Jaćimovic, 2022). Herbal medicine has proved virtuous and persuasive in the treatment of many chronic diseases that conventional medicine cannot cure. Plants also have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. The use of herbs to treat disease is almost ubiquitous among non industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals (Kumar, Loveleena, Godwin, 2013).

Plant phytonutrients are a rich source of important drugs used in degenerative diseases. Today we are witnessing a great deal of public interest in the use of herbal remedies. Ethno pharmacological studies of medicinally important plants have attracted the investigators throughout the world. One such plant *Murraya koenigii* has invited the attention of researches worldwide for its biological activities. A very common member of our household gardens, commonly known as "lemon basil", its local names are efirin oso in Yoruba and marugbo sanyan in Hausa. It is an aromatic, more or less deciduous shrub or a small tree up to 6 meters in height, found throughout Nigeria up to an altitude of 1500 meters and cultivated mostly for its aromatic leaves (Ahmad, Tan, Hazni, & Nafiah 2015).

SPECIFIC OBJECTIVES

1. To analyze the phytochemical contents of *Murraya koenigii* leaves.
2. To analyze the proximate content of *Murraya koenigii* leaves.
3. To determine the effect of *Murraya koenigii* leaves extract on bodyweight changes and any adverse effects in hyperlipidemia induced wistar rats
4. To assess the impact of *Murraya koenigii* extract on lipid parameters (such as Total Cholesterol (TC), Triglycerides, Low Density Lipoprotein-Cholesterol (LDL-C), Very Low Density Lipoprotein-Cholesterol (VLDL-C) and High Density Lipoprotein Cholesterol (HDL-C)) in hyperlipidemia induced wistar rats.

JUSTIFICATION OF THE STUDY

There are several justifications for evaluating the effects of *Murraya koenigii* on dyslipidemia in Wistar rats. Firstly, *Murraya koenigii* (curry leaves) has been used in traditional medicine for its various health benefits including its potential to improve lipid profile. Investigating its effects on dyslipidemia in a controlled animal study can help validate traditional use. Animal studies serve as a crucial step in screening potential therapeutic agents before human clinical trials. Assessing the effects of *Murraya koenigii* on dyslipidemia

in Wistar rats can provide valuable preclinical data to determine its efficacy and safety. Wistar rats are commonly used in dyslipidemia research due to their genetic predisposition to develop metabolic disorders making them a suitable model for studying lipid metabolism. Evaluating *Murraya koenigii* in this model can help understand its mechanisms of action on dyslipidemia. Previous studies have shown that *Murraya koenigii* exhibits antioxidant, anti-inflammatory and hyperlipidemic properties. Conducting a systematic evaluation in a standardized animal model like Wistar rats can contribute scientific evidence supporting its potential as a therapeutic agent for dyslipidemia. Lastly, dyslipidemia is a major risk factor for cardiovascular diseases, which are leading causes of morbidity and mortality worldwide. If *Murraya koenigii* shows promising effects on dyslipidemia in animal studies, it could offer a natural and accessible treatment option with potential public health benefits.

PLANT PROFILE

Gahlawat *et al.* (2014) provided the following profile for the plant as follows; Kingdom: Plantae, Subkingdom: Tracheobionta, Super division: Spermatophyta, Division: Magnoliopsida, Subclass: Rosidae, Order: Sapindales. Family: Rutaceae, Genus: *Murraya* J.Koenig ex L., Species: *Murraya koenigii* L. Spreng.

Table 2.1: Nutritional Value

S.N.	Nutrients	Value of fresh curry leaves(100gm)	Value of dehydrated curry leaves(100gm)
1.	Proteins	6 g	12 g
2.	Fat	1 g	5.4 g
3.	Carbohydrate	18.7 g	64.31 g
4.	Calcium	830 mg	2040 mg
5.	Iron	0.93 mg	12 mg
6.	β carotene	7560 μg	5292 μg

Source: Plant, Singh, More & Madan, (2015).

Phatak, Khanwelkar, Datkhile & Hendre (2018) stated *Murraya koenigii* is a small deciduous aromatic shrub reaching to the height of about 6-9m grown 1500m above sea level. Main stem is dark green to brown colored bark with several dots on it that can be flaked off lengthways to expose the white wood beneath. The leaves are 15-30 cm long bipinnately compound supporting 11-25 leaflets, leathery and glandular. Leaves are bitter in taste, slightly acidic and feebly pungent (Sablania, Basak, & Bosco, 2023). The leaves are shown in the figure 2.1.



Figure 2.1: *Murraya koenigii* Leaves

Source: Phatak *et al.* (2018)

Flowers are sweet odor, funnel shaped, inflorescence bearing 60-90 flowers, bisexual, complete and stalked with diameter 1.12 cm when fully opened with 4-5 sepals and 10 straight stamens. The flowering starts from mid-April to mid-May whereas fruiting occurs from mid-July to end of August. It is a self-pollinated crop (3. 2 3, n.d.). Fruit is edible black berry each with 1.4 to 1.6 cm long and 1 to 1.2 cm diameter with shiny surface containing 0.76% of yellow volatile oil. The fruits occur in close bundles containing 32-80 fruits per bundle (Gahlawat *et al.*, 2014). Roots are extensively laid out and are woody giving rise to suckers. The propagation is generally done with seed but root suckers and air layering can also be used and germination can occur in partial shade (Chauhan, Dedania, Mashru., 2017). Curry Leaf plant is true diploid with chromosome number 18 (Bhopal, Buchineni, Kudagi, Pathapati, Haritha, & Anjani, 2016).

2.4 MORPHOLOGY AND PHYSIOLOGY OF *MURRAYA KOENIGII*

Curry leaf plants are small to medium-sized evergreen trees, typically growing up to 4-6 meters in height. The leaves are usually dark green, glossy, and pinnate, consisting of 11-21 leaflets arranged in pairs along a central stem (Chute & Dakhane, 2022). The plant produces small white flowers in clusters, which eventually develop into small, shiny, black berries. Roots of *Murraya koenigii* are fibrous and shallow (Curry & Williams, 2022).

Curry leaf plants require warm, tropical climates to thrive and are sensitive to frost. They prefer well-draining, fertile soil with regular watering but can tolerate brief periods of drought (Chute & Dakhane, 2022). The plant is known for its aromatic leaves, which contain essential oils responsible for its distinctive flavor. Curry leaves are rich in nutrients like iron, calcium, vitamin C and Anti-oxidants, making them a popular ingredient in traditional medicine and culinary practices. They are hardy plant with unique foliage and valuable medicinal and culinary properties (Curry & Williams, 2022).

TRADITIONAL USES OF *MURRAYA KOENIGII*

Curry leaf plant is popular among South Asian Dishes for its peculiar taste and aroma. It has been used as a home remedy since ages (Chauhan, Dedani & Mashru, 2017). The scented leaves are widely used in flavoring curries to promote appetite and digestion (Kataria, Singh, Gupta, Jalhan, & Jindal, 2013). Leaves

are locally used to treat external injuries, burns and remove poison from the bite of poisonous animals and for treating rheumatism (Tan *et al.*, 2014). Baked (cooked, crisped) leaves are used to check vomiting (Kataria *et al.*, 2013). Finely grinded leaves mixed in butter milk have positive effects for stomach upsets and act as laxative when taken in an empty stomach (Kataria *et al.* 2013). Fresh leaves juice mixed with lime and sugar is used to treat morning sickness and root juice consumption gives renal pain relief (Nishan & Subramanian, 2015). Stem is used to cleanse teeth that lead to reinforcing the gums (Widayanti, Srifiana & Efend. 2019). Fruit has anti-astringency properties. Root juice is used in kidney pain. Curry leaf can be used in treating calcium, vitamin deficiencies and anemia. Moreover antitumor, hypoglycemia, antihyper-cholesterol emic effects of the plant has been found (Kumari and Papiya, 2014). Piles, body heat, inflammation and itching are controlled with curry leaves (Das, 2022). Traditional Ayurveda includes the use of curry leaf parts as a cure of cough, hypertension, hepatitis, rheumatism and hysteria (Ghasemzadeh, Jaafar, Rahmat & Devarajan, 2014). Traditionally curry leaves are boiled together with coconut oil until reduction to blanked residue to be used as hair tonic for keeping natural hair tone and invigorating growth of hair.

MATERIALS AND METHODS DRUGS AND CHEMICALS

S/N	DRUGS AND CHEMICALS	MANUFACTURER
1.	Metformin	Emzor Pharmaceuticals Nig. Ltd
2.	Atorvastatin	Emzor Pharmaceuticals Nig. Ltd
3.	Fructose	Dangote Group
4.	Butter	Dangote Group
5.	Triglyceride reagent	Falcon Chemicals
6.	Glycerol	Falcon Chemicals

Metformin and Atorvastatin as pure powder were purchased from a store in Owerri. Fructose was obtained as well from a store in Owerri, Imo state. , butter (vanaspati ghee), egg yolk and coconut oil were procured from the Econuwa market in Owerri Imo state. All reagents and chemicals used were of analytical grade and stored in a refrigerator at -4°C. The reagents were also be equilibrated at room temperature for 30 min before analysis.

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

Fresh curry leaves '*Murraya Koenigii*' was obtained from a garden located in Owerri-North Local Government Area, Imo State, Nigeria, and then certified and authenticated by Prof. F.N Mbagwu, a taxonomist in the Imo State University Owerri. The plant specimen was deposited in the herbarium. Then the fresh curry leaves were washed under tap water thoroughly; dried under shade and powdered by using a mechanical grinder.

PREPARATION OF *Murraya koenigii* LEAVES EXTRACTS

Methanolic and aqueous extracts of *Murraya koenigii* leaves were prepared by soxhletation method. About 50g of shade dried leaves powder of *Murraya koenigii* were defatted using petroleum ether; dried, then packed in a cloth bag and placed in the thistle of Soxhlet apparatus. The Soxhlet continuous extraction process continued for 4 days till the dark brown colour of aqueous extract turned to pale yellow while in the case of methanolic extract, the appearance of dark green to colorless. Collected extracts were then concentrated in the vacuum rotary evaporator and dried by evaporating in hot air oven at 45°C. Percentage

yield of Methanolic Extract of *Murraya Koenigii* (MEMK) and Aqueous Extract of *Murraya Koenigii* (AEMK) were calculated with respect to the total quantity of powder used for the extraction.

ACUTE TOXICITY STUDY OF EXTRACTS

AEMK and MEMK were performed in the acute toxicity test as per OECD-423 guidelines 10 for fixing the therapeutic dose. The dose of 2000 mg/kg b w of AEMK and MEMK was taken as a starting dose and orally administered to two healthy Wistar rats. LD50 was determined and 1/10th of LD50 was taken as therapeutic dose for the activity.

EXPERIMENTAL DESIGN AND METHODS

Thirty-six healthy Wistar rats of male sex were randomly divided into six groups (six animals per group) and they had free access to water and animal diet throughout the study period. For the pilot study, ten healthy Wistar rats of male sex were selected; their blood samples were collected by retro-orbital puncture method; Blood Sugar Level (BSL) and lipid profile were assessed.

INDUCTION OF HYPERLIPIDEMIA

Hyperlipidemia in rats was induced through HFFD model. Hyperlipidemia model were developed using a high fructose (10% fructose) added in drinking water bottle and high fat (butter (vanaspati ghee): coconut oil in ratio of 3:1) mixed with an egg yolk in dough of animal food diet. HFFD were fed to rats of Group 1 to 6 (Except Group 3) throughout the study period of 14 weeks.

GROUPING OF ANIMALS

Group 1-AEMK: Rats received HFFD and administrated AEMK-200 mg/kg/day, p. o

Group 2-MEMK: Rats received HFFD and administrated MEMK-200 mg/kg/day, p. o

Group 3-NC: Rats received normal diet and served as normal control

Group 4-MET: Rats received HFFD and administrated Metformin-50 mg/kg/day, p. o **Group 5-ATO:** Rats received HFFD and administrated Atorvastatin-10 mg/kg/day, p. o **Group 6-HC:** Rats received HFFD and served as hyperlipidemia control.

STATISTICAL ANALYSIS

All values were expressed as mean and standard deviation. Data were analyzed by two-way analysis of variance (ANOVA) and significant differences between groups were determined by Duncan's multiple range tests and Least Significant Difference (LSD). Statistical analyses were done using SPSS, the statical package for widows, version 11.0 (SPSS Inc. Chicago, IL USA).

RESULTS AND DISCUSSIONS PERCENTAGE YIELD

Percentage yield was found to be AEMK (14.35%) and MEMK (13.75%) with respect to the total quantity of powder used for the extraction.

ACUTE TOXICITY STUDY OF EXTRACTS

The acute toxicity study observed no mortality or any toxic reactions within 4h and after 14 days by oral administration of AEMK and MEMK even at the highest dose (2000 mg/kg). The dose of 200 mg/kg was considered safe at 1/10th dose of LD50.

PHYTOCHEMICAL AND PROXIMATE ANALYSIS OF ETHANOL EXTRACT OF MURRAYA KOENIGI Table

4.1: Phytochemical Analysis of *Murraya Koenigii* Leave Extract

PHYTOCHEMICALS	CONCENTRATION (MG/100 G)
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ALKALOIDS	344.34±0.26
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CYANOGENIC GLYCOSIDES	11.08±0.34
PHENOLICS	1136.78±0.36
SAPONINS	0.03±0.01
FLAVONOIDS	601.25±0.43
TANNINS	206.05±7.5
	0.11±0.06
CAROTENOIDS	

Note; Data are means of triplicate determinations on a dry weight basis ± standard deviation

Table 4.2: **Proximate Composition of *Murraya Koenigii* Leave Extract**

VARIABLE	CONTENT (%)
CARBOHYDRATES	1.29±0.02
PROTEINS	3.61±1.29
FATS AND OILS	5.14±0.96
CRUDE FIBRE	1.78±0.52
ASH	3.60±0.22
MOISTURE	84.61±1.21

Note: Data are means of triplicate determinations on dry weight basis ± standard deviations **PILOT STUDY OF BSL AND LIPID PROFILE**

Body weight, BSL and lipid profile of ten rats were assessed. BSL and lipid profile were established for the normal range reference as shown in Table 1. All values are shown which is in mostly agreement with the results of study by Ahangarpour et al. (2016).

Table 4.3: **Baseline values of glycemic and lipid profile of normal rats in the pilot study.**

Rats	BSL (mg/dL)	TC (mg/dL)	TG (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	AIP
Mean	78.2	56.5	41.71	8.34	34.5	13.68	0.17

Min	44	46	14	3	5.12	3	-0.314
Max	124	86	92	18	57	30.7	0.9
Range	40-124	40-90	10-100	2-20	5-57	3-30	-0.3-0.9

BW-Body weight, BSL-Blood sugar level, TC-Total cholesterol, TG-Triglycerides, LDL-C-Low density lipoprotein cholesterol, HDL-C-High density lipoprotein cholesterol, VLDL-C-Very Lowdensity lipoprotein cholesterol, AIP-Atherogenic index of plasma.

EFFECT OF MURRAYA KOENIGII LEAVES EXTRACTS ON BODY WEIGHT

Body weight was calculated on first and final days of the experimental study period. This is shown in the Table 4.4 below.

Table 4.4: Effect of *Murraya koenigii* leaves on body weight in HFFD fed rats.

Group	Initial body weight (g on first day)	Final body weight (g on last day)	Percentage change in body weight
I-NC	108.02 ± 07.38	203.83 ± 04.93	46.60 ± 04.98
II-HC	123.33 ± 08.63	227.17 ± 08.69	46.92 ± 02.64
III-AEMK	122.85 ± 10.46	138.16 ± 12.45***	11.17 ± 01.41**
IV-MEMK	112.01 ± 17.53	208.33 ± 06.25NS	46.47 ± 10.02NS
V-MET	104.33 ± 04.49	166.83 ± 04.49***	3.44 ± 03.77
VI-ATO	136.00 ± 10.83	169.84 ± 05.86***	18.02 ± 08.80*
ANOVA	F	1.247	18.353
	P	0.3119	< 0.0001***
			0.0003

*Values are expressed in Mean ± SEM, Number of animals = 4, NS- Not significant, ***- p<0.001, **- p <0.01, *- p <0.05 (Comparison with VI), ATO- Atorvastatin (10 mg/kg/day), MET- Metformin(50 mg/kg/day), AEMK- Aqueous extract of *Murraya koenigii* leaves (200 mg/kg/day), MEMK -Methanolic extract of *Murraya koenigii* leaves (200 mg/kg/day).*

Extremely significant effect on body weight (p < 0.001) in Groups I, IV and V while no significant change were observed in Group II when compared to group VI. Percentage change in body weight was found highly significant change (p < 0.01) in Group I, significant change (p < 0.05) in Group V whereas no significant change were observed in Group II when compared to Group VI.

DISCUSSIONS

In this research study, both of AEMK and MEMK have shown hypolipidemic effects in HFFDfed rats. HFFD pattern for the period of 14 weeks could be responsible to induce elevated levels of TC, TG and LDL-C in comparison to the normal control group. Both of AEMK and MEMK have exhibited an important role in lowering levels of TC and LDL-C in compared to group VI remarkably. According to Phatak et al., (2019), *Murraya koenigii* leaves has proven as potent free radical scavenger in the DPPH assay so that bioactive compounds are involved in depleting TC levels through two possible mechanisms i.e. reducing fat absorption in the digestive system and increasing fat excretion into faeces.

Furthermore, our findings in this research study have shown that there was no significant change in BSL in the 1st and 14th days of experimental study period which is in consistency with the study reported by Lozano et al. (2016). Our results of the study has indicated that HFFD model does not induce hyperglycemia in the period of 14 weeks which is in agreement with the study reported by Zaman et al. (2011).

According to study by Lozano et al. (2016), it is suggested that HFFD model needs more than 6 months period to provoke hyperglycemia. It shows similar lipid lowering effect in spite of different solvents used for extraction like chloroform, dichloromethane, ethyl acetate and ethanol apart from aqueous and methanol extracts. Other than leaves, stem bark extract of *Murraya koenigii* has shown to exert hypolipidemic effect which is similar to that effect of leaves. Hypolipidemic effect of AEMK is in agreement with the aqueous extract of *Murraya koenigii* in the studies of Kesari et al. (2005), Upadhye et al. (2014), Ekoh et al. (2014), El-Amin et al. (2013), Lawal et al. (2008), Vinuthan et al. (2007) while MEMK is in conformity with the methanolic extract of this plant in study by Vinuthan et al. (2007).

CONCLUSION

Murraya koenigii is a verdant therapeutic as well as green verdant plant that has a place with family Rutaceae. The different pharmacological exercises of the plant has been seen, for example, movement on Enemy of diabetic, cholesterol diminishing property, antimicrobial action, antiulcer action, Antioxidative property, cytotoxic action, hostile to looseness of the bowels action, against malignant growth action with numerous other phagocytic action. The synthetic structure of the *Murraya koenigii* comprises of natural ointment alkaloids and terpenoid. Subsequently Curry leaves justify further phytochemical, pharmacological and clinical examinations for advancement of a viable normal plant.

Results of the present study have suggested that the antihyperlipidemic activity of *Murraya koenigii* leaves leading to decrease serum lipid parameters mainly TC, LDL-C along with atherogenic risk is due to the presence of its bioactive compounds.

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