International Journal of Scientific Research Updates

Journal homepage: https://orionjournals.com/ijsru/

ISSN: 2783-0160 (Online)

(RESEARCH ARTICLE)

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Effect of ethanolic leaves extract of *Myrianthus arboreus* on lipid profile of Wistar rats

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International Journal of Scientific Research Updates, 2024, 07(02), 001–008

Publication history: Received on 07 February 2024; revised on 18 March 2024; accepted on 21 March 2024

Article DOI: https://doi.org/10.53430/ijsru.2024.7.2.0029

Abstract

This study investigated the effect of ethanolic leaves extract of *Myrianthus arboreus* on lipid profile of Wistar rats. A total of 24 albino Wistar rats divided into 8 groups of three rats each, were orally administered with DMSO as control and varying doses of 1500 mg/KgBw, 1000 mg/KgBw and 500 mg/KgBw for 7days and 14 days respectively. After 7 and 14 days groups 1-4 and 5-8 were sacrificed respectively. Blood samples and hearts of rats were collected for biochemical and histopathology investigations respectively. The result showed non-significant differences ($p>0.05$) in the mean values of total-cholesterol (T-Chol) and Low density lipoprotein-cholesterol (LDL-Chol.) in all the extract treated groups for 7 and 14 days when compared to mean control values. Mean values of triglyceride (Tg) and very low density lipoprotein-cholesterol (VLDL-Chol) significantly increased ($p<0.05$) in 1500 and 1000 mg/kgbw extract treated groups for 7days and decreased significantly ($p<0.05$) in 500 mg/kgbw for 14 days when compared with mean control values. The results also reveal a significant decrease ($p<0.05$) in the level of high density lipoprotein-cholesterolHDL-Chol) for groups treated 1500 1000 and 500 mg/kgbws for 14 days when compared with control. Phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins and tannins with phenols and tannins having the highest and lowest concentrations of 1185.04 and 16.75 mg/100g respectively. The histopathology results showed normal histology of the heart tissues of animals in groups 1,3-8 while that of group 2 showed inflammation of the heart tissues. In conclusion, oral administration of ethanol leaves extract of *Myrianthus arboreus* is safe for the heart.

Keywords: *Myrianthus arboreus*; Heart; Phytochemical; Lipid profile; Triglyceride

1. Introduction

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date [1]. Plants are the source of medication for preventive, curative, protective or promotive purposes [2]. Different plant parts and components (roots, leaves, stem, barks, flowers and their combinations, essential oils) have been employed in the treatment of infectious pathologies and respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin [3-4]. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [5]. Several groups of constituents in plants have been identified as potentially health promoting in animal studies including cholesterol lowering factors, antioxidants, enzyme inducers and others [6]. The plants are applied in different forms such as poultices, concoction of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridge, soup administered in different ways including oral, nasal (smoking, snuffing or steaming), topical (lotions, oils or creams) bathing or rectal (enemas) [1].

An estimated 400 million inhabitants of the world, that is about 80% of world's population, are thought to rely chiefly on traditional medicine, which is largely of plant origin, for their primary health care needs [7]. However, it is widely believed that these valuable medicinal resources in plants are largely untapped because of inadequate scientific technical and commercial infrastructures in developing countries [8]. The relatively cheaper cost of medicinal plants

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and its availability in Africa have made them attractive as therapeutic alternative when compared to modern medicines because of their antimicrobial properties [9-11]. In recent years, there is a growing interest in herbal therapy. The major contributory factors to this growing interest include: rising costs of orthodox medications, low therapeutic index of synthetic compounds and the growing incidence of drug resistance [12].

Myrianthus arboreus belongs to the family Moraceae. It is a monoecious forest regrowth tree up to 15m high with a characteristic false fruit which is yellow when ripe. Leaves are very large, alternately shaped, digitately 5-7 foliolate. Young leaves are usually red in colour. Male inflorescences are yellow, branched and produced panicle like axillary pairs towards later part of the dry season. The female inflorescences are paired, stalked, greenish clusters (pendunculate). Fruit is syncarpous and basally fused, yellow drupes up to 10cm with stylar remains projecting from each drupe. The leaves are used in preparation to treat dysentery, diarrhea and vomiting. In eastern Nigeria plaster made of beaten leaf are applied to boil . Chopped leaves are eaten raw with salt for heart problems and pregnancy complications. Sap from the leaves is applied tropically for toothache, to the chest for bronchitis or for sore throat [13]. In Rumuji community of Rivers State, the plant is called 'Uzere' and is used as an analgestic. In West Africa young leaves are eaten in vegetable soups. In Delta and Edo States of Nigeria, the leaves of *Myrianthus arboreus* are rated among the most popular indigenous vegetables. Throughout the range of the species, the heart shaped fruit, called 'God's heart' in Ghana, is eaten for its sweet or acidulous pulp. The oilrich seed, which is about 1 cm long, is eaten after cooking from Côte d'Ivoire to DR Congo [14].

2. Materials and methods

2.1. Collection and Identification of Plant

The leaves of *Myrianthus arboreus* were collected from a forest near Our Saviours Chapel, University Of Port Harcourt. It was then deposited at the Herbarium unit of Department of Plant Science and Biotechnology for authentication.

The plant was identified by Dr. Nwosu Edwin. The herbarium number was given as UPH/V/1,244.

2.2. Apparatus and Equipment's

Spectrophotometer (surgispec 5m-23d; surgifield medicals, england), Rotary Evaporator (RE52A, England Lab Science), Analytical Balance (HCK LN 0708), Water Bath (TT-6 Techmel &Techmel USA), Centrifuge (Universal 320 laboratory century Hettich Zentrifugen), refrigerator (Frestech),

2.3. Chemicals And Reagents*:*

All chemicals and reagents used in this study were of analytical grade.

2.4. Extract Preparation

The fresh leaves of *Myrianthus arboreus* were washed with tap water and air dried for two weeks. The dried plants were then pulverized into powdered form using an electric blender. A coarse powdered material was obtained, macerated using 99.7% ethanol in a 5000ml maceration jar and left to stand for three days (72 hours). The mixture was gently shaken daily for the three days. After the three days period, the mixture was filtered first using a handkerchief and thereafter a Whatman filter paper. Rotary evaporator was used to concentrate the filtrate and recover the ethanol from the solution, a gel like extract which was placed in a crucible in a water bath at a temperature of 40^0C for 24hours was obtained. The extract was then stored in a refrigerator pending usage.

2.5. Source of experimental animals

Thirty (30) wistar rats of both sexes were purchased from the animal house of the Department of Anatomy, College of Basic Medical Sciences, University Of Port Harcourt. The animals were kept and worked on in the same Animal House. The rats were grouped based on weight difference of ±5g in a group. The Department of Biochemistry Research Ethics Committee at its emergency meeting held on the 25th March, 2024, considered the application for ethical approval and after due deliberations gave approval with reference UPH/BCHREC/2024/017A

2.6. Lethal Dose (LD_{50}) Determination

The LD_{50} was carried out using the method described by the Organization for Economic Cooperation and development (OECD) guidelines for testing of chemicals. Three doses of 1000 mg/kgBW, 3000mg/kgBW, 5000mg/kgBW were orally administered to 6rats divided into three groups of two rats per group. The rats were then observed for 24hours and for

seven (7days). No death was recorded therefore safe doses of 1500 mg/kgBW, 1000 mg/kgBW and 500 mg/kgBW were selected for the research.

2.7. Experimental design

24 rats were divided into 8 groups of 3 rats each. 1ml of DMSO was orally administered to rats in groups 1 and 5 for 7 and 14 days respectively serving as vehicle control, while 1500 mg/kg, 1000 mg/kg and 500 mg/kg of ethanolic leaves extract of *Myrianthus arboreus* dissolved in DMSO were orally administered to rats in groups 2- 4 and 6-8 for 7 and 14 days respectively.

2.8. Sacrifice of Animals

Rats were anaesthetized by placing in a desiccators containing cotton wool soaked with chloroform. Rats in groups 1-4 and 5-8 were sacrificed and dissected after 7 and 14th day of extract administration and blood collected into heparinized sample bottles while hearts were stored in universal bottles preserved with 10% formalin.

2.9. Biochemical Investigation

Total Cholesterol (TC) and Triacylglycerol (TG), were estimated by enzymatic methods described by Allain *et al.* [15] using assay kits (Randox Laboratories Ltd, UK). High Density Lipoprotein Cholesterol (HDL-C) was determined by enzymatic method described by Stein [16] using assay kits (Randox Laboratories Ltd, UK). Low-Density Lipoprotein Cholesterol (LDL-C) was calculated using the formular by Friedewald *et al.* [17].

2.10. Histopathogical Examination

Hearts from treated and control groups were fixed in 10% freshly prepared formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin and eosin [18].

2.11. Statistical Analysis

Statistical analysis was carried out using SPSS version 21 (IBM). The data were analyzed using one way analysis of variance (ANOVA) and significant difference were determined using Post Hoc and Turkey's test for multiple comparison at $p<0.05$. All data were expressed in mean ± standard deviation (M±SD)

3. Results

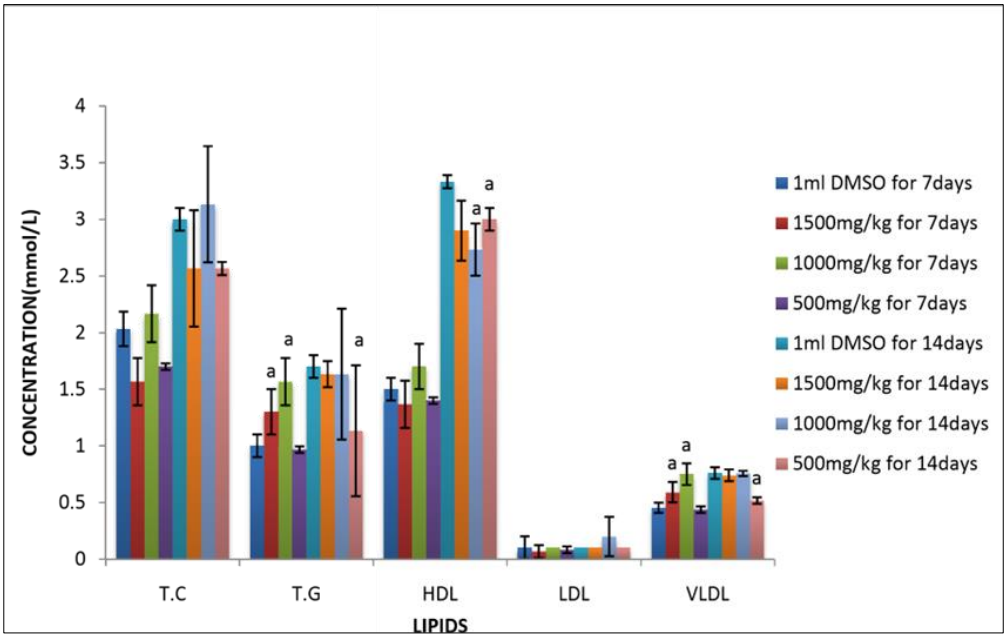

Figure 1 Effect of Ethanolic Leaves Extract of *Myrianthus arboreus* on Lipid Profile of Wistar Rats. Superscript "a" indicates significant difference ($p<0.05$) when compared to control group. N=3

Table 1 Quantitative phytochemical screening of *Myrianthus arboreus*

Names of phytochemicals	Concentration mg/100 g
Alkaloids	297.38
Flavonoids	19.63
Glycosides	20.96
Phenols	1185.04
Saponins	52.28
Tannins	16.75

3.1. Photomicrograph of Heart tissues

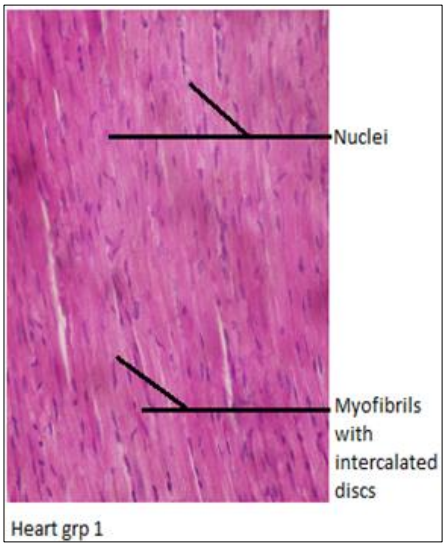

Figure 2 Photomicrograph of heart tissues of rats giving DMSO as vehicle control for 7days (H and E STAIN) X400. Result revealed no change in heart architecture

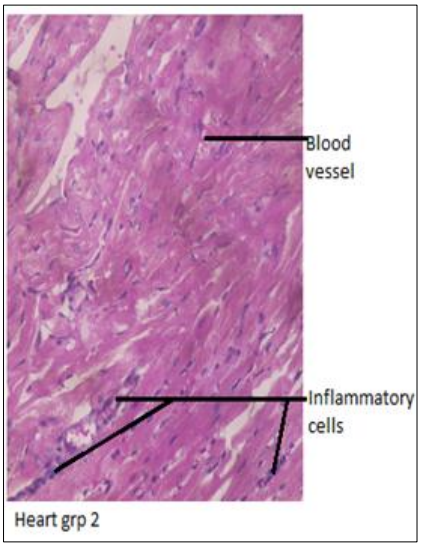

Figure 3 Photomicrograph of heart tissues of rats administered with 1500 mg/kg for 7days (H and E STAIN) X400. Result revealed inflammation of heart tissues

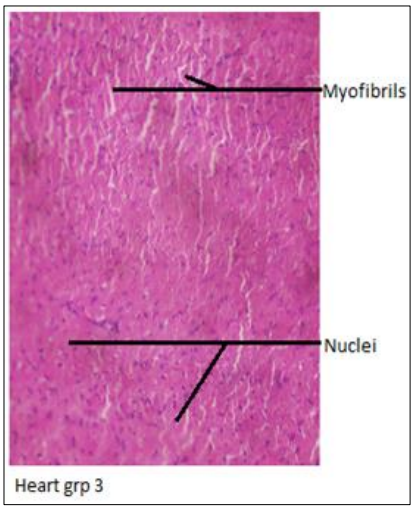

Figure **4** Photomicrograph of heart tissues of rats administered with 1000 mg/kg for 7days (H and E STAIN) X400. Result revealed normal heart architecture

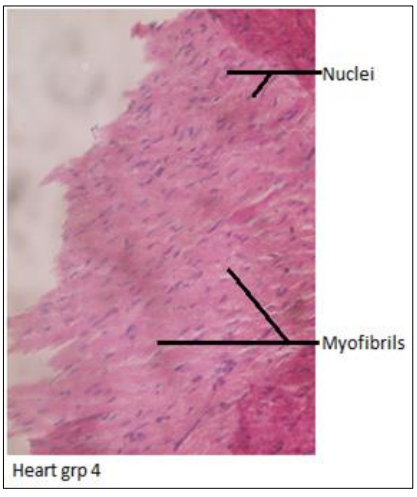

Figure **5** Photomicrograph of heart tissues of rats administered with 500 mg/kg for 7days (H and E STAIN) X400. Result revealed normal renal architecture

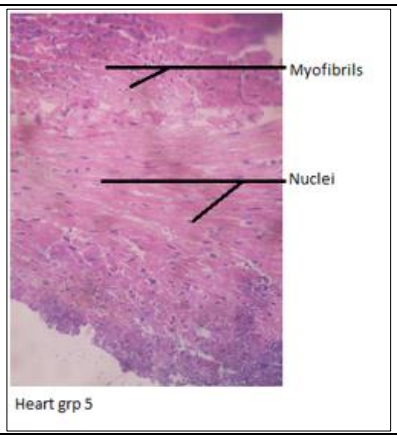

Figure 6 Photomicrograph of heart tissues of rats giving DMSO as vehicle control for 14 days (H and E STAIN) X400. Result revealed no change in heart architecture

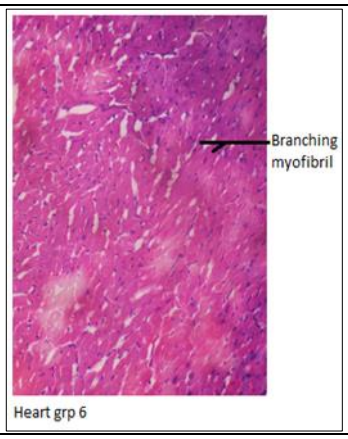

Figure 7 Photomicrograph of heart tissues of rats administered with 1500 mg/kg for 14 days (H and E STAIN) X400. Result revealed normal heart architecture

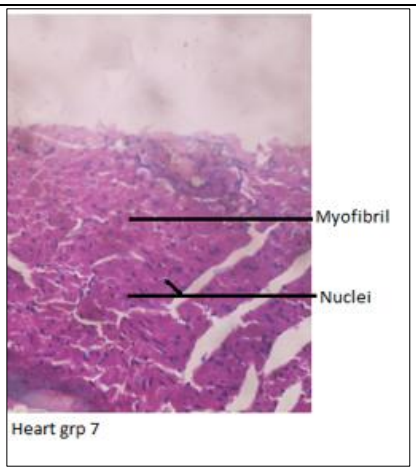

Figure 8 Photomicrograph of heart tissues of rats administered with 1000 mg/kg for 14 days (H and E STAIN) X400. Result revealed normal heart architecture

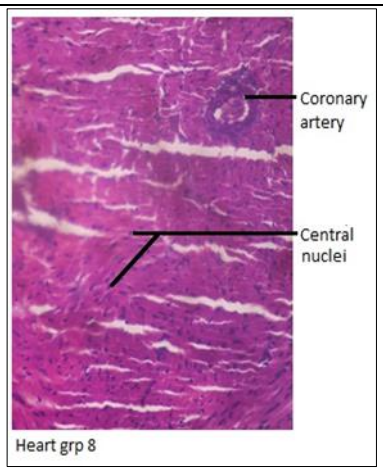

Figure 9 Photomicrograph of heart tissues of rats administered with 500 mg/kg for 14 days (H and E STAIN) X400. Result revealed normal heart architecture

4. Discussion

Cardiovascular disease (CVD) accounts for about 30% of all deaths worldwide making health care systems expensive [19-20]. Abnormal lipid metabolism is one of the major factors that cause cardiovascular disease and particularly heart disease [21]. High levels of triglycerides (TG), low-density lipoprotein (LDL) and cholesterol are markers of CVD [22-24]. Impact of different phytochemical compositions on lipid profile and markers of CVD risk is recently the focus of research [25].

The result in Fig.1 showed non significant differences ($p>0.05$) in the mean values of totalcholesterol (T-Chol) and Low density lipoprotein-cholesterol(LDL-Chol.) in all the extract treated groups for 7 and 14 days when compared to mean control values indicated reduction in cardiovascular risk. Jacobson [26] reported that best indicator of atherosclerosis risk is elevated low density lipoprotein cholesterol (LDL). For many years, cholesterol has been directly related to cardiovascular prognosis [27] and related increase in total cholesterol to increase in the risk of coronary-related mortality. International Lipid Information Bureau, [28] emphasized the importance of the LDL fraction and that the cardiovascular risk may best be defined by the magnitude of the LDL-cholesterol rather than its total cholesterol.

Mean values of triglyceride (Tg) and very low density lipoprotein-cholesterol (VLDL-Chol) significantly increased ($p<0.05$) in 1500 and 1000 mg/kgbw extract treated groups for 7days and decreased significantly ($p<0.05$) in 500 mg/kgbw for 14 days when compared to mean control values. VLDL is a TG -rich lipoproteins, several studies have indicating that high levels of triglycerides leads to increase in the risk of cardiovascular disease in men and in women

[23,29-31]. The results also reveal a significant decrease ($p<0.05$) in the level of high density lipoproteincholesterol (HDL-Chol) for groups treated 1500, 1000 and 500 mg/kgbws for 14 days when compared with control. HDL-cholesterol, has inverse relationship with the risk of coronary heart disease because of it's reverse cholesterol transport as well as anti-inflammatory capacity and protection against LDL-cholesterol oxidation [32]. Decreased HDL-Chol level indicates high cardiovascular disease risk.

Phytochemical screening of the plant revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins and tannins with phenols and tannins having the highest and lowest concentrations of 1185.04 and 16.75 mg/100g respectively. Several researchers reported saponin's biological fnctions as anti-inflammatory, anti-diabetic, anti-HIV, antiatherosclerotic and serve as protective functions like gastro-protective, hepatoprotective, hypolipidemic [33-36].

The histopathology results showed normal histology of the heart tissues of animals in group 1,3-8 while that of group 2 showed inflammation of the heart tissues.

5. Conclusion

In conclusion, oral administration of ethanolic leaves extract of *Myrianthus arboreus* is safe to the heart.

Compliance with ethical standards

Acknowledgments

This research was not sponsored by any organization or institution (self-sponsored)

Disclosure of conflict of interest

No conflicting interest existed among authors of this work.

Statement of ethical approval

The Department of Biochemistry Research Ethics Committee at its emergency meeting held on the 25th March, 2024, considered the application for ethical approval and after due deliberations gave approval with reference UPH/BCHREC/2024/017A.

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