**Investigating the cardio-protective activity of *Volvariella volvacea* in isoproterenol-induced myocardial infarction using animal model: An *in vivo* approach**

## ABSTRACT

Ischemic heart disease (IHD) is a severe cardiovascular condition characterized by impaired blood flow to the heart muscle, leading to tissue damage and dysfunction. Among different natural resource, mushrooms are now becoming more attractive because of its strong nutritional value and therapeutic potentiality. Wild edible fleshy fungi like *Volvariella volvacea* has been instrumental in preventing and treating chrome diseases. Medicinal plants abundant in our environment have been instrumental in preventing and treating chronic diseases, especially cardiovascular disorders. Hence, this study was carried out to evaluate the cardio-protective activity of *Volvariella volvacea* in isoproterenol-induced myocardial infarction using animal model. Twenty five (25) male wistar rats were randomly grouped into five classes (A, B, C, D and E) of five rats each. Groups A, B and C were pre-treated with 100mg/kg, 200mg/kg and 400mg/kg of *Volvariella volvacea* extract respectively. Group D was administered 10 mg of lisinopril (standard drug), this served as the positive control group while group E was administered 0.2 ml distilled water, and this served as the negative control group. All the rats were intraperitoneally induced with 150 mg of isoproterenol after fourteen days (two weeks) of pretreatment. Cardiac biomarker (lactate dehydrogenase (LDH)), was measured to assess cardiac injury, using standard method while lipid bioassay such as high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG) and total cholesterol (TC) were assayed using standard assay kits. Results showed that rats pre-treated with *Volvariella volvacea* extract showed significant effect on cardiac biomarkers compared to the control group. Results showed a significant increase ($P<0.05$) in high density lipoproteins (HDL) levels and lactate dehydrogenase (LDH) activities with a significant decrease ($P>0.05$) in low density lipoproteins (LDL) and triglyceride levels in a dose-dependent manner (100<200<400 mg/kg body weight) among the extract treated groups when compared to the untreated control group. However, total cholesterol levels of extract treated groups showed no significant difference when compared with the untreated control group. This study has shown that extract of *Volvariella volvacea* could help to mitigate cardiovascular diseases and could be used to produce plant based products to combat Ischemic heart disease, and consequently improving general wellbeing.

**Keywords:** *Volvariella volvacea*, Ischemic heart disease, therapeutic purposes, myocardium.



## INTRODUCTION

Ischemic heart disease (IHD) is a spectrum of cardiovascular disorders with untold high morbidity and mortality and remains the major and leading cause of disability worldwide [1]. It ranges from simple angina through unstable angina, myocardial infarction and sudden cardiac death. It results from progressive reduction in coronary blood flow with resultant imbalance between demand and supply ratio of nutrients to myocardial tissue. Myocardial infarction (MI) which results from complete cessation of blood flow to the myocardium is among the leading cause of death and the most severe clinical presentation of coronary artery disease (CAD) [2]. Its presentation could be in form of ST elevation myocardial infarction (STEMI) and Non-ST Elevation Myocardial Infarction (NSTEMI). Unstable angina typically presenting as NSTEMI may potentially progress to STEMI. Both conditions are therefore referred to as Acute Coronary Syndrome (ACS) [3, 4]. More than 3 million individuals present as STEMI each year, but, post mortem studies showed a higher figure of more than 4 million as many may die on the spot or on transit to hospital [5].

MI commonly has a high prevalence among the middle-aged individuals and this invariably has a high economic impact in the global economy. Recently, more than 1.1 million hospitalizations following MI were reported in the United States, with an estimated direct cost of $450 billion [6].

Isoproterenol (ISO) administration in experimental animals provided a rapid, simple, and noninvasive method to generate myocardial damage similar to that seen in humans with acute MI. In addition, ISO produced a model that had low mortality, high reproducibility, and validity compared with other animal models, which make it more appropriate for the assessment of potential cardio-protective agents [7]. The main mechanism involved in isoproterenol-induction of myocardial ischemia is the generation of free radicals, reactive oxygen species, lipid peroxidation, oxidative stress, and calcium overload, which lead to the alteration in membrane permeability, causing apoptosis and necrosis and finally slowing the conduction between myocardial cells, triggering alterations in heart electrical activity [8]. Diverse bioactive agents extracted from natural resources have been targets for new drug discovery and have established a wide variety of biological actions [9] especially in combating myocardial infarction.

Mushrooms have become one of the most important sources of functional food and medicines in recent years [10]. The demand for edible mushrooms has increased due to their taste, flavor, and nutrient content [11, 12]. Mushrooms are better alternatives to animal proteins and other animal products, and this fact has been supported through various studies conducted in the past [13, 14].

Several forms of vitamins in mushrooms are responsible for improving health by decreasing the risk of various diseases in humans [15].

*Volvariella volvacea* is one of the largest genera of macrofungi, with several edible species that have medicinal and high nutritional values [15]. *V. volvacea*, a member of the Agaricaceae family, ranks at the top among cultivated mushrooms, and it is well known for its edibility. It is considered as one of the most important mushrooms based on its culinary and medicinal values [16]. Production and consumption of this mushroom have been consistently increasing for the last six to seven decades, with China ranking highest in production and exports to Russia, Japan, Vietnam, Korea, and Thailand, as well as several European and African countries [16, 17]. Several important bioactive compounds have been isolated from *V. volvacea* during the past few years [18] and these have contributed significantly to their medicinal properties [16, 19]. Recent evidence suggests that *V. volvacea* contains high levels of substances of possible medicinal importance, among which includes tyrosinase, aromatase inhibitors and immunemodulating as well as antitumor properties [20]. Researchers have also shown that cold water extracts of *V. volvacea* have a geno-protective activity [20]. Arising from the above, *V. volvacea*, like other mushrooms, is considered to be valuable component of human diet, especially by health-conscious people. This suggests that the value of *V. volvacea* as a medicinal substance warrants more detailed study. Therefore, the objective of this study is to investigate the cardio-protective activity of *V. volvacea* on myocardial infarction through the induction of isoproterenol. This study will not only further explore some mechanisms underlying the cardio-protective effect of *V. volvacea* by evaluating the expressions and activities of cardiac and antioxidant biomarkers but also cost-effective means of preventing or reducing the burden of myocardial infarction in human.



## MATERIALS AND METHODS

### Sample Collection and Preparation

Fresh sample of *Volvariella volvacea* were purchased from a market at Asaba, Delta State, Nigeria. Identification and authentication of the fungi was carried out at the Department of Botany, Nnamdi Azikiwe University, Awka and a voucher specimen was deposited at the herbarium of the Department for future references. Sporophores, solitary or gregarious, centrally stipitate, growing on heaps of rotten paddy straw. Pileus 3.5 - 10.5 cm in diameter, campanulate when young, becoming umbonate at maturity, brown, dark brown at the umbo. Gills crowed distinctly formed free, thin, reddish at maturity. Spore print brown. Stipe 5.0 - 10.0 cm long, whitish, solid bulbous base, volva well developed, persistent, bilobed, membranous and margin free, annulus absent. Flesh white, soft. Hymenophoral trama inverse, Basidia, clavate, tetrasterigmatic. (18.5 - 28.0 X 6.5 - 11.5) µm. Basidiospores (6.5 - 9.0 X 4.5 - 6.2) µm, oval to ovoid, smooth, thin wall. Pleurocystidia and Cheilocystidia present. The samples were thereafter shredded with a knife and oven-dried at 40$^oC$ in order to get rid of the moisture as well as preserve the bioactive compounds. The dried sample was pulverized using a laboratory blender and the fine powders obtained was weighed and stored in an air-tight container at room temperature for further use.

**Extraction of Plant Materials**

The weighed powdered sample (200.60 g) was then used for the extraction with a solvent combination of ethanol and water (7:3) (2000 ml) for 72 hr via maceration in an unheated medium. The mixture was decanted and filtered using sterile Whatman paper No. 1. The filtrate was there after evaporated to dryness with the aid of a rotary evaporator set at 50 $^oC$ to obtain crude ethanol extract which was carefully preserved for further analysis. The method of Nkafamiya *et al* [21] was used to calculate the yield (10.05 g) of the crude extract using the formula below:

$$\text{Percentage yield} = \frac{Mass\ of\ crude\ extract\ (g)}{Mass\ of\ powdered\ sample\ (g)} \times 100$$

**Animal Studies**

**Procurement of study animals**

Wistar albino rats (30) weighing approximately 180 g were purchased from Chris Farm Ltd Mgbakwu, Awka, Anambra State and were brought to the animal house of the Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The rats were kept in standard cages with saw dust as bedding, and at standard room temperature as well as standard housing conditions of 12:12 light: dark cycles and fed with standard rat pellets and water *ad libitum*. The animals were allowed to acclimatize to the new environment for seven days.

**Dose preparation and treatment**

The hydro-ethanolic extract of *V. volvacea* was prepared with distilled water in three divided doses (100, 200, and 400) mg / kg, lisinopril (10 mg/kg) was used as a reference drug and distilled water was used as a vehicle for the untreated group. The animals were administered the extract and drug orally for fourteen consecutive days concurrently prior to the induction with water *per os* and feed *ad libitum*.

**Experimental design**

The animals were randomly grouped into five, with six animals in each group, and the treatment was as follows: Groups A, B and C animals were designated as *V. volvacea* treatment group and were pre-treated with the ethanol extract at 100 mg/kg, 200 mg/kg and 400 mg/kg, respectively, for 14 days and thereafter 0.2 ml isoproterenol (ISO) at 150 mg/kg was injected intraperitoneally at an interval of 24 h on the 15th and 16th day. Group D animals were designated as isoproterenol control and were administered 0.2 ml of 10 mg lisinopril for 14 days and thereafter 0.2 ml isoproterenol (ISO) at 150 mg/kg was injected intraperitoneally at an interval of 24 h on the 15th and 16th day while group E animals (designated as vehicle control group) were administered 0.2 ml distilled water for 14 days; and on the 15th and 16th day, 0.2 ml isoproterenol (ISO) at 150 mg/kg was injected intraperitoneally at an interval of 24 h.

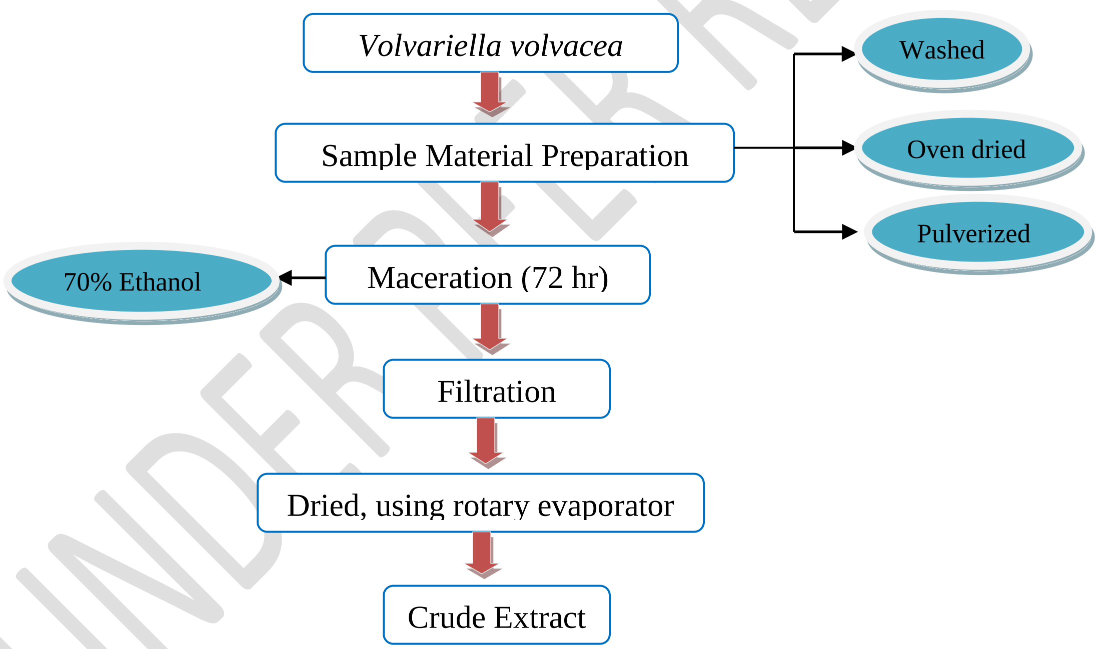


Fig. 1: Procedure for the extraction of *Volvariella volvacea* crude extract

**Collection of blood sample**

At the end of the experimental period, the animals were anesthetized with chloroform vapor and sacrificed. A 5 ml sterile syringe with needle was used for blood collection through cardiac puncture and the sera obtained were used for bioassay studies.

**Biochemical assays**

Assay of creatinine kinase (CK-MB), lactate dehydrogenase (LDH), aspirate aminotransferase (AST) and alanine aminotransferase (ALT) activities were carried out with standard assay kit sourced from Randox laboratories Ltd, United Kingdom with maximum adherence to the manufacturer's instruction.

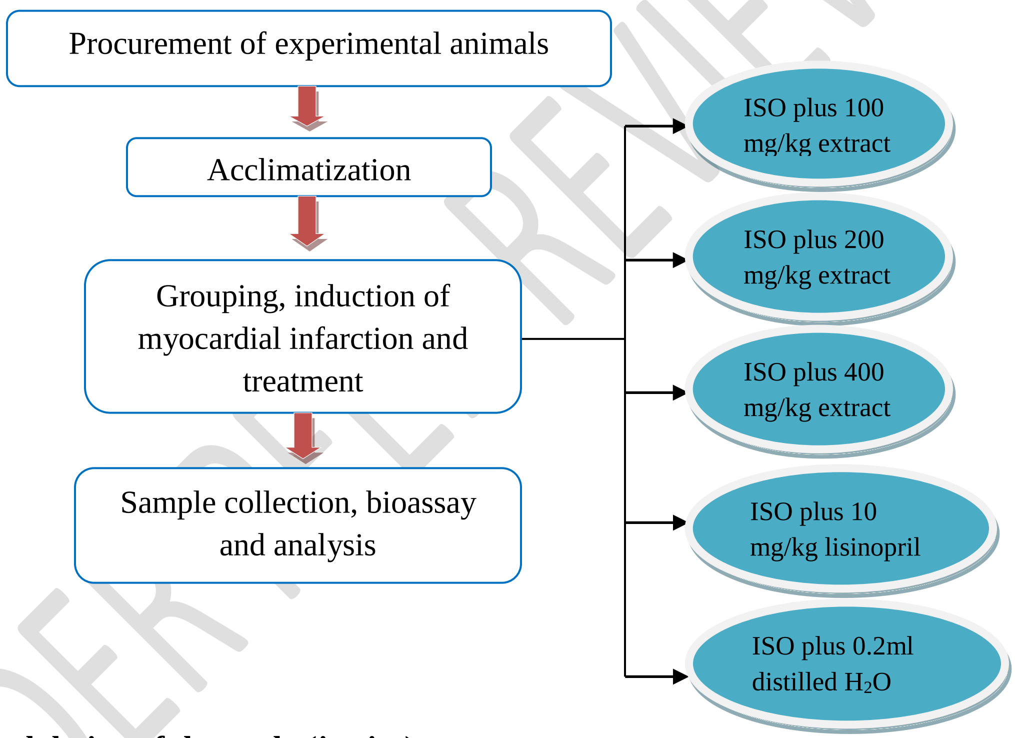


**Fig. 2: Experimental design of the study (*in vivo*)**

**Data Analysis**

The results obtained in this research were expressed as Mean ± SEM of triplicate determinations within each group. One-way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p<0.05$. GraphPad Prism5 Program (GraphPad Software, San Diego, CA, USA) was used for the graphical analyses of the results obtained.

**RESULTS**

The result showing the effect of oral administration of the fruiting body extract of *Volvariella volvacea* on high density lipoprotein (HDL) levels in wistar rats induced with acute myocardial infarction is presented in figure 1. Result showed a significant increase ($p<0.05$) in HDL levels in extract treated group in a dose-dependent manner (100<200<400 mg/kg) body weights when compared with the control groups.

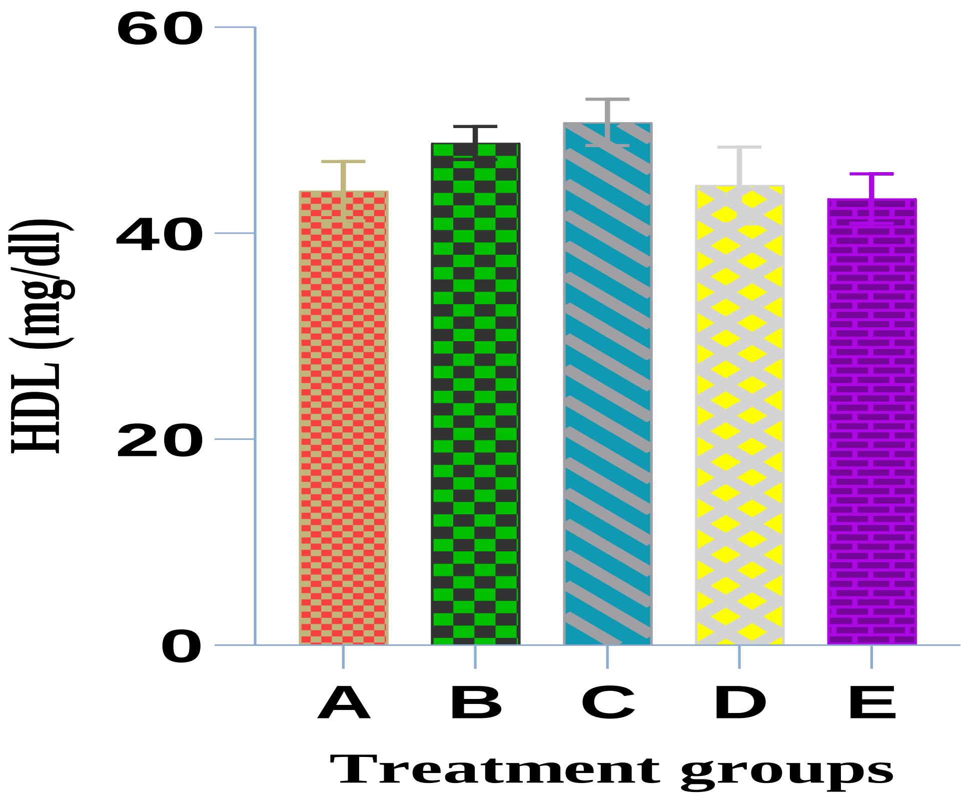


**Figure 3: Effect of oral administration of the fruiting body extract of *Volvariella volvacea* on lactate high density lipoprotein (HDL) levels in wistar rats induced with acute myocardial infarction**

Figure 4 presents the effect of oral administration of the fruiting body extract of *Volvariella volvacea* on low density lipoprotein (LDL) levels in wistar rats induced with acute myocardial infarction. Result showed a significant increase ($p<0.05$) in LDL level of untreated control (group D) when compared with the extract treated group.

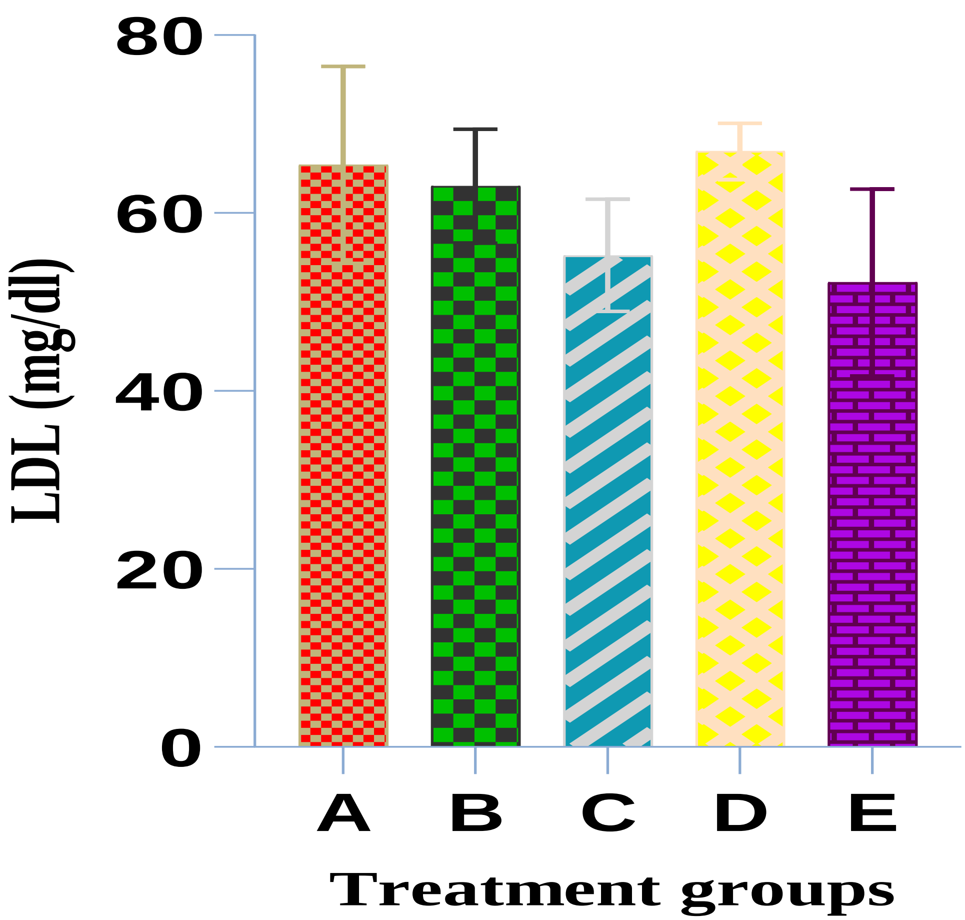


**Figure 4: Effect of oral administration of the fruiting body extract of *Volvariella volvacea* on low density lipoprotein (LDL) levels in wistar rats induced with acute myocardial infarction**

The result showing the effect of oral administration of the **fruiting body** extract of *Volvariella volvacea* on triglyceride (TG) levels in wistar rats induced with acute myocardial infarction is presented in figure 5. Result showed a significant increase ($p<0.05$) in triglyceride levels of untreated animals (group D) when compared with the extract-treated groups.



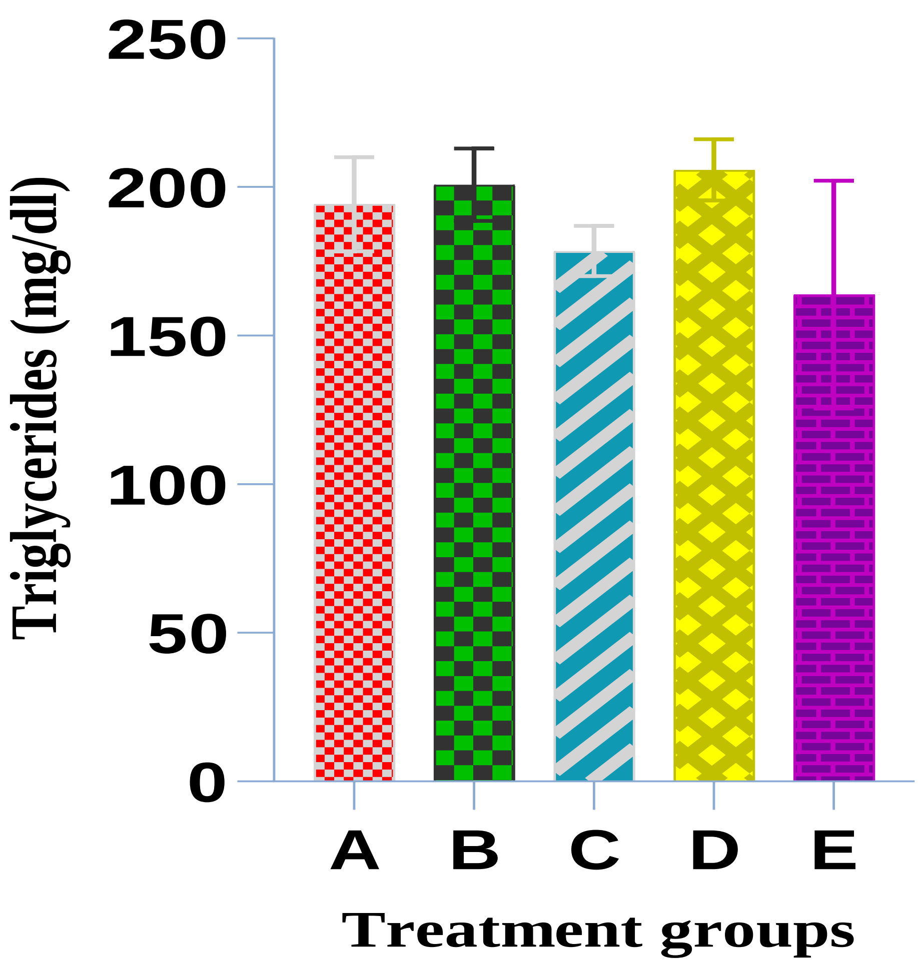


**Figure 5: Effect of oral administration of the fruiting body extract of *Volvariella volvacea* on triglyceride (TG) levels in wistar rats induced with acute myocardial infarction**

Figure 6 presents the effect of oral administration of the **fruiting body** extract of *Volvariella volvacea* on total cholesterol (TC) concentration in wistar rats induced with acute myocardial infarction. Result showed no significant difference in total cholesterol levels of extract treated groups in a when compared with the negative control (group D).

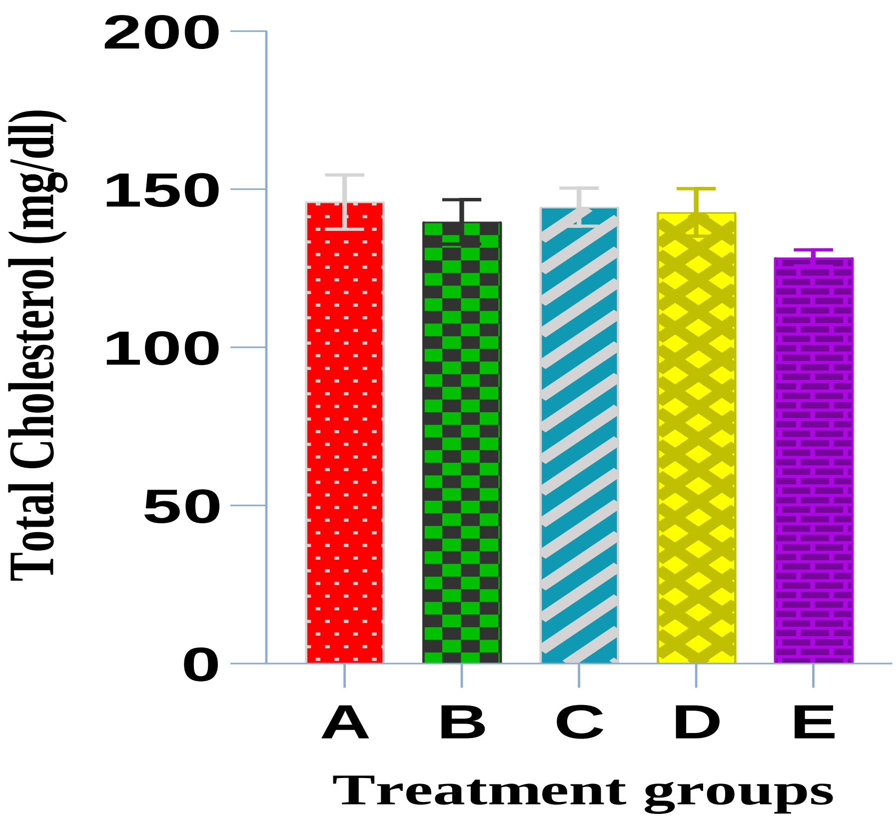


**Figure 6: Effect of oral administration of the fruiting body extract of *Volvariella volvacea* on total cholesterol (TC) levels in wistar rats induced with acute myocardial infarction**

The result showing the effect of oral administration of the leaf extract of *Volvariella volvacea* on lactate dehydrogenase (LDH) activities in wistar rats induced with acute myocardial infarction is presented in figure 7. Result showed a significant increase ($p<0.05$) in LDH activities in extract treated group in a dose-dependent manner (100<200<400 mg/kg) body weights when compared with the control groups.

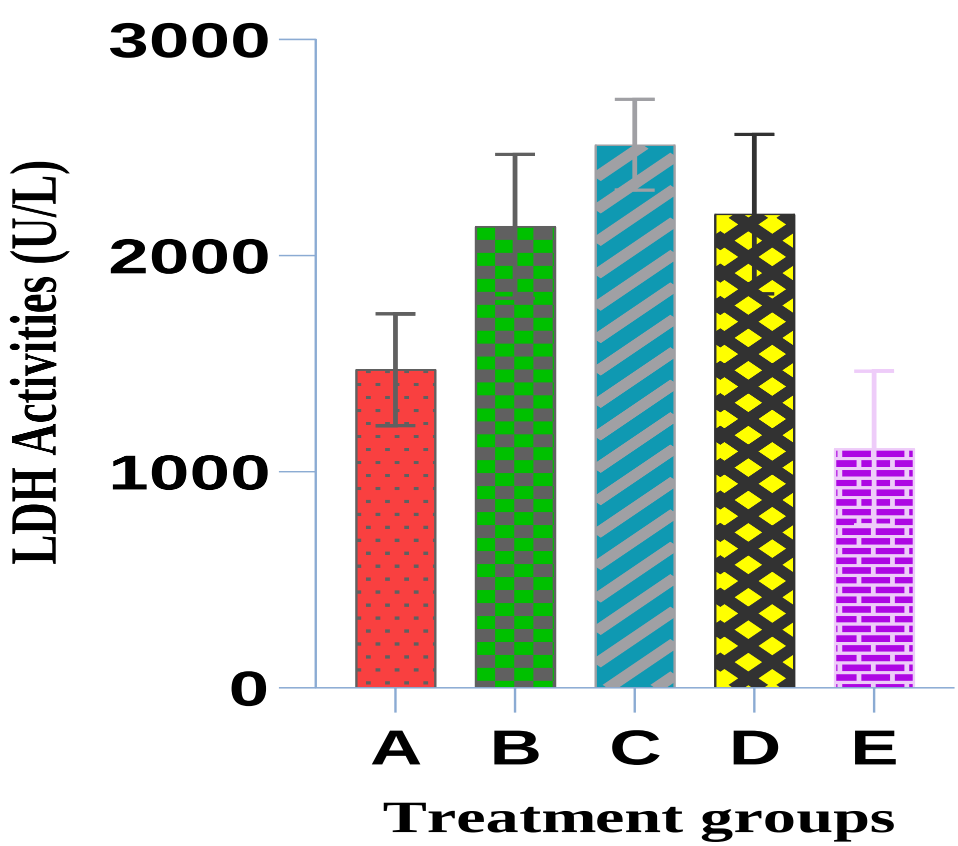


**Figure 7: Effect of oral administration of the leaf extract of *Volvariella volvacea* on lactate dehydrogenase (LDH) activities in wistar rats induced with acute myocardial infarction**

**Discussion**

The use of plants in the treatment of various ailments especially cardiovascular diseases is gaining wide acceptance globally [2, 22]. This has been chiefly attributed to the presence of a number of bioactive compounds inherent in them [3, 23]. Evaluating the effects of plants' administration on myocardial infarction remains vital in evaluating their therapeutic index against other cardiovascular diseases. Hence, investigating the protective ability of *Volvariella volvacea* in isoproterenol-induced myocardial infarction using animal would be a sterling way to addressing the perennial problems associated with cardiac disorders.

The results from this study showed that **fruiting body** extract of *Volvariella volvacea* could help to ameliorate cardiovascular disorders especially myocardial infarction. The result showing the effect of oral administration of the **fruiting body** extract of *Volvariella volvacea* on high density lipoprotein (HDL) levels in wistar rats induced with acute myocardial infarction is presented in figure 1. Result showed a significant increase ($p<0.05$) in serum HDL levels in extract treated groups (A, B and C) in dose-dependent manner (100<200<400 mg/kg) body weights when compared with the control groups (D and E). This is an important observation because HDL has been shown to play a significant role in scavenging cholestereol and its esters from the blood and peripheral tissues; breaking them down to bile acids and consequently disabling the formation of atherosclerotic plague [2, 24]. This could possibly be due to the increasing activity of lecithin-cholesterol acyl transferase (LCAT), an enzyme responsible for incorporating free cholesterol into HDL, thereby promoting reverse cholesterol transport and competitively inhibiting the uptake of LDL; and thus protecting the walls of the artery from clogging thereby preventing myocardial infarction. Gao *et al*, [25] made similar assertion.

Low density lipoprotein (LDL) is is often referred to as the "bad cholesterol" because it plays a significant role in the pathogenesis of myocardial infarction. Research has shown that LDL is responsible for transporting about 70% of total cholesterol to the body cells. Hence, an increase in total cholesterol level consequently increases LDL. Furthermore, reports from epidemiologic, genetic and clinical studies have undoubtedly shown that increased serum LDL is the primary cause of atherosclerotic disorder [26].

From the result of this study (fig. 2), **fruiting body** extract of *Volvariella volvacea* was able to significantly reduce the serum LDL levels in the treated grroups and consequently reduces the possibility of cardiovascular disorder especially myocardial infarction when compared to the untreated group. Previous studies, especially Khatana *et al*., [27] has reported that *V. volvacea* contained some phenolics which may work by increasing LDL receptors densities in the liver binding to apo-lipoprotein B thereby making liver cells more efficient to remove LDL from blood. This is consistent with the reports of Onwubuya and Oladejo [3].

Another important parameter worthy of note is the triglyceride. They are any of a group of lipids that are esters formed from one molecule of glycerol and three molecules of one or more fatty acids, and are widespread in adipose tissue, which commonly circulate in the blood in the form of lipoproteins. Triglycerides are an important measure of heart health as its uncontrolled increase have been shown to be one of the risk factors of heart attacks, strokes as well as liver and pancreas problems. Sufficient evidences have shown that serum triglyceride level above 150 mg/dL in growing adult may elevate the risk of arteriosclerosis (hardening of the arteries or thickening of the artery walls), heart attack, stroke, and other heart disease. Evidently, as shown in this study (Fig. 3), induction of isoproterenol significantly increases the serum triglyceride levels in the experimental animals; however, treatment with the extract of *V. volvacea* was able to mitigate this continued increase in a dose-dependent manner (400 mg/kg < 200 mg/kg < 100 mg/kg), thereby exerting protective effect on the cardiomyocytes. . Sikarwar and Patil, [28] and Onwubuya *et al*., [2] in their separate studies reported that plant extract have the ability to activate the endothelium bound lipoprotein lipase which hydrolyses triglyceride into fatty acid and therefore decreasing triglyceride levels.

Conversely, the result in Figure 6 showed no significant changes in total cholesterol levels of the treated group when compared to the untreated group. Total cholesterol has been defined as the cumulative measurement of cholesterol content in the bloodstream, encompassing both high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, with the normal range to be less than 200 mg/dL, borderline high as 200 to 239 mg/dL and high at or above 240 mg/dL (. The lack of significant changes in total cholesterol levels might suggest that the primary effects of *V. volvacea* extract are more targeted towards specific lipoprotein components rather than a broad spectrum cholesterol-lowering effect. This selective modulation could be particularly advantageous by avoiding the potential negative effects of excessively low cholesterol levels, such as hormonal imbalances and cognitive issues [29, 30].

As presented in Figure 7, there was an increase in lactate dehydrogenase (LDH) activity in the untreated group when compared to the treated group, in a dose-dependent manner (100<200<400 mg/kg). The increase in LDH activity observed in our study suggests an enhancement of cellular metabolism or a response mechanism to myocardial damage. LDH is an indicator of tissue damage and its elevation typically reflects ongoing cellular repair processes. The increase in LDH levels in response to the extract treatment could indicate enhanced turnover and repair of myocardial tissue, possibly facilitated by bioactive components of the extract such as its polysaccharide bioactivity, which could have protective effects against ischemic damage. This polysaccharide bioactive compound, particularly beta-glucans, has been demonstrated to modulate immune responses and cellular metabolism. Beta-glucans have been shown to trigger cellular activity, potentially causing an increase in LDH release as a consequence of enhanced metabolic processes [31].

## 5. CONCLUSION

The findings of this research have provided evidence for the cardioprotective efficacy of *V. volvacea* extract against myocardial infarction. This can be drawn from its significant modulation of key biochemical parameters, including increasing high-density lipoprotein (HDL), decreasing low-density lipoprotein (LDL), particularly in the prevention and management of lipid-associated disorders, as well as triglycerides (TG). Cardioprotective effect was also achieved by inducing an adaptive response in lactate dehydrogenase (LDH) activity, underscoring its potential as a versatile therapeutic agent for cardiovascular health. Hence, *V. volvacea* could serve as a form of amelioration to cardiovascular diseases, specifically myocardial infarction.

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