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Biochemical evaluation on amelioration of oxidative stress and cardiovascular risk markers in hyperlipidemic rats treated with *Kalanchoe pinnata* aqueous extract

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Abstract. *Odinga-Israel TB, Lemii BC, Gabriel-Brisibe CU, Daka IR, Enebeli SK, Austin-Asomeji I, Ucheawaji F, Nodi CC. 2024. Biochemical evaluation on amelioration of oxidative stress and cardiovascular risk markers in hyperlipidemic rats treated with* Kalanchoe pinnata *aqueous extract. Asian J Nat Prod Biochem 22: 98-105.* The continuous use of plants as a raw material for medicines has gained wide acceptance due to their pharmacological bioactive compounds. Metabolic disorders such as hyperlipidemia could result in cardiovascular diseases. This study evaluated the effect of the aqueous extract of *Kalanchoe pinnata* (Lam.) Pers. on the antioxidant, lipid profile, and clinical indices in hyperlipidemic female albino rats. The female albino rats were induced with hyperlipidemia using a high-fat diet mixture consisting of 68% powdered rat feed (standard rat chow pellet), 20% instant milk powder (peak milk), 6% corn oil (Mazola), and 6% ghee. Thirty female albino rats were grouped into 5, with 6 rats in each group. Group 1 served as normal control, group 2 was positive control, and group 3 was negative control, while groups 4 and 5 were administered 200 mg/kg and 400 mg/kg aqueous *K. pinnata* extract for 21 days. The body weight of the rats was recorded after 21 days. The rats were then sacrificed, and blood samples were collected to determine the antioxidant status, lipid profile, troponin, CK, and myoglobin levels of the rats using standard laboratory procedures. *K. pinnata* extract showed significant antioxidant and antihyperlipidemic activities, with the most at 400 mg/kg, evidenced by a decrease in the TC, TG, and LDLC serum levels with a corresponding increase in the HDLC serum level for anti-hyperlipidemia. An increase in the GSH, GST, GPx, catalase, and SOD levels was observed with a corresponding decrease in MDA serum levels in the experimental rats. Although myoglobin, troponin, and CK are not acutely evaluated for myocardial infarction, they can be used for deduction on biochemical disorders. The serum concentrations of myoglobin, CK, and troponin were decreased on administration of *K. pinnata* when compared to the control groups. The findings of this study suggest that *K. pinnata* could be used as a raw material for the medicinal treatment of cardiovascular diseases and as an antioxidant source.

Keywords: Antioxidant, anti-hyperlipidemia, cardiovascular markers, *Kalanchoe pinnata*, myoglobin, troponin

INTRODUCTION

Biochemical disorders are disruptions or imbalances in the body's normal metabolic processes. These types of disorders are related to the development of various health conditions, including cardiovascular diseases, high cholesterol levels (hyperlipidemia), and diabetes mellitus (Barr 2018). These biochemical disorders increase disease susceptibility. Therefore, proper medical screening and monitoring are necessary to help prevent or manage the onset of these related health conditions. Research has indicated that alterations in antioxidant status and increases in inflammatory markers are often associated with metabolic syndromes and their various components. As a result, those diagnosed with metabolic syndrome are more prone to oxidative stress. It lowers antioxidant levels to compensate for the elevated Reactive Oxygen Species (ROS) levels (Suriyaprom et al. 2019).

The use of natural products from plants has gained attention. It can be attributed to the potency of plants, including their ethnomedicinal value, due to their phytochemical content and biologically active compounds (Odinga et al. 2016). The use of plants for therapeutic, prophylactic, and ameliorative purposes has been encouraged because plants are cost-efficient and readily available (Odinga et al. 2020).

In line with the words of Hippocrates in 400 BC, "Let food be thy medicine and medicine thy food," many plants around our environment serve as food sources and have health benefits such as *Kalanchoe pinnata* (Lam.) Pers.. It is native to Madagascar, an herbaceous perennial, reasonably abundant along the coast, growing in sandy soils. It is used mainly in African, Brazilian, and Indian

traditional medicine for treating several diseases such as diabetes (Nascimento et al. 2023). The crude extracts contained several phytochemical compounds, including tannins (Zawirska-Wojtasiak et al. 2019). It has anthelmintic activity (Muzitano et al. 2006), a protective effect against stomach lesions or ulcers induced by various factors, including aspirin, indomethacin (a type of nonsteroidal anti-inflammatory drug), serotonin, reserpine, stress, ethanol (alcohol) (de Araújo et al. 2018). A study by Richwagen et al. (2019) found that 60% methanolic leaf extract of *K. pinnata* at a concentration of 25 mg/mL inhibited the growth of *Bacillus subtilis, Shigella dysenteriae, Escherichia coli, Proteus vulgaris,* and *Staphylococcus aureus,* but not against, *Klebsiella pneumoniae, Candida albicans,* and *Pseudomonas aeruginosa*.

Hyperlipidemia could arise from an unhealthy diet and lack of exercise and physical activity. This condition can contribute to the buildup of plaque deposits on the inner walls of blood vessels. Over time, the plaque clogs the arteries, leading to high blood pressure and increasing the risk of stroke and cardiovascular diseases such as heart attacks. Therefore, proper diet and regular exercise help prevent and manage hyperlipidemia and its health consequences (Alloubani et al. 2021). Numerous traditional and folkloric plant applications have been documented in peer-reviewed scientific literature. However, further investigation in the Niger Delta region of Nigeria revealed that the local population consumes the plant in a concoction with malt and milk. This traditional preparation is utilized to manage lipid profiles in the blood and enhance overall blood health. Given the absence of prior scientific research on the reported traditional uses of K. pinnata, this study aimed to investigate its effects on antioxidant status, lipid profile, and specific biochemical markers in female albino rats induced hyperlipidemia. The goal was to provide scientific proof of the claimed benefits associated with using K. pinnata for lipid management and related health outcomes.

MATERIALS AND METHODS

Plant collection and preparation of extract

Fresh leaves of *K. pinnata* were harvested in the Emoh community in the Abua/Odual local government area, Rivers State, Nigeria, in September 2022. Healthy and mature leaves were used in this study. The sample was authenticated by Dr. M. Ajuru of the Department of Plant Science and Biotechnology, Rivers State University, Nigeria (voucher number SUK 5279). Two kg of plant sample was ground by a mechanical grinding machine and then macerated with water (3 L) for 24 hours. After filtration and lyophilization, 59.2 g of extract was obtained. The solution (1 g/mL) was prepared by dissolving the extract in distilled water freshly each time before use for administration. The extracts were stored until use at 4°C.

Animals for experiment

Thirty adult female albino rats weighing 250 to 300 g were obtained from the Department of Pharmacology and Therapeutics, Rivers State University, Port Harcourt, Nigeria and were used in this study.

Preparation of standard drug (simvastatin)

Simvastatin was obtained from a commercial Pharmacy in Port Harcourt. Simvastatin (20 mg) was dissolved in 12.5 mL of normal saline in a beaker for a 1.6 mg/mL concentration.

Administration of standard drug (simvastatin)

The standard drug simvastatin was prepared daily and administered using an oral gavage tube to the experimental animals in group 3 as a positive control.

Induction of hyperlipidemia

The female rats with an average body weight of 250 to 300 g were induced hyperlipidemia using a High-Fat Diet (HFD) for 7 days. The composition of the high-fat diet following Kadir et al. (2015) contained 414.0 kcal/100 g, with a composition of 43% carbohydrates, 40% fat, and 17% protein (Table 1). The diets were a mixture of 68% powdered rat feed (standard rat chow pellet), 20% instant milk powder (peak milk), 6% corn oil (Mazola), and 6% ghee (popularly known as *manshanu* in Northern Nigeria). All ingredients for high-fat feed are mixed thoroughly and baked at 65°C in an oven overnight. A standard rat chow diet contains 306.2 kcal/100 g, with 3% fat, 21% protein, and 48.8% carbohydrate. Hyperlipidemia was confirmed by measuring rats' lipoproteins and serum lipids levels.

Experimental design

The animals were weighed before the experiment, observed for physical symptoms, and recorded. The rats were acclimatized for 7 days and were fed ad libitum. The treatment procedure was as follows*:* Thirty (30) albino rats weighing 250-300 g were grouped into 5 groups, with 6 rats in each group. (i) Group 1: Normal Control group (standard feed + water); (ii) Group 2: Negative Control Group (HFD + water); (iii) Group 3: Positive Control Group (HFD + simvastatin (standard drug) + water); (iv) Group 4: 200mg/kg *K. pinnata* extract (HFD + 200 mg/kg *K. pinnata* + water); (v) Group 5: 400mg/kg *K. pinnata* extract (HFD + 400 mg/kg *K. pinnata* + water).

Table 1. Composition of a high-fat diet (Kadir et al. 2015)

High-fat diet	
Nutrients	**%/100 g**
Carbohydrate	43
Protein	17
Fat	40
Ingredients	**g/100 g**
Powdered rat feed	68.0
Maize oil	6.0
Ghee	6.0
Milk powder	20.0
Total energy (kcal/100 g)	414.0

Administration of extracts

Group 4 was treated with *K. pinnata* extract at 200 mg/kg BW and 400 mg/kg BW for group 5 using oral gavage for 21 days.

Body weight gain

The impact of treatments on body weight gain was assessed weekly on each rat using an electronic weighing balance (KERN 440-35 N) throughout the study period. The % mean body weight difference was calculated using the formula as described by Odinga et al. (2023):

$$\% \text{ mean body weight difference} = \frac{Final\ weight - Initial\ weight}{initial\ weight} \times 100$$

Sample collection for biochemical analysis

The experiment lasted for 21 days, after which animals were sacrificed. At the end of the treatment, the animals were fasted for twenty-four hours. The rats were put in a desiccator and allowed to anesthetize slightly following the absence of oxygen, and blood samples were collected by jugular venipuncture. The blood samples (2 mL) were collected into sterile plain sample bottles for each rat, agitated slowly, and appropriately covered. The blood samples were analyzed for antioxidant biomarkers, i.e., Reduced Glutathione (GSH), Glutathione-S-transferase (GST), Glutathione Peroxidase (GPx), Catalase, Superoxide Dismutase (SOD), Malondialdehyde (MDA)), lipid profile (Triglyceride (TG), Total Cholesterol (TC), Low-Density Lipoprotein (LDL), High Density Lipoprotein (HDL)), troponin, creatine kinase, and myoglobin.

Determination of antioxidant biomarkers

The antioxidant activity of *K. pinnata* aqueous leaf extract was evaluated by measuring the antioxidant markers in the serum of the experimental albino rats.

Estimation of malondialdehyde

The malondialdehyde level was calculated using the method of Bahekar and Kale (2016). The malondialdehyde (MDA) level in the plasma samples was measured to indicate lipid peroxidation. MDA is one of the aldehyde products formed during lipid peroxidation. It reacts with thiobarbituric acid (TBA) to produce a colored product. The absorbance of this colored complex was measured spectrophotometrically at 530 nm. 0.5 mL of serum was taken and put in test tubes, added with 3 mL of 10% trichloroacetic acid (TCA), mixed well, and the tubes were left to stand at room temperature for 10 minutes. The tubes were centrifuged for 15 minutes at 5,000 rpm, and 2 sets of test tubes were prepared - one for the blank and one for the test sample. For the test sample, 2 mL of the supernatant was added with 1.5 mL of 0.67% TBA. For the blank, 2 mL of distilled water was added with 2 mL of 0.67% TBA. The tubes were mixed well and then placed in a boiling water bath for 10 minutes; a pale pink color developed after cooling under tap water. The color intensity was measured using a colorimeter at 530 nm. The MDA concentration was calculated by the molar extinction coefficient 1.5×10^5 and expressed as nmol of MDA per 100 mL of serum.

1.5 = 100 µmol/L (here, 100 is conversion from mL to dL)

Then MDA = 100 × OD of unknown/1.5

Where: O.D = Optical density

Estimation of superoxide dismutase

Superoxide dismutase was estimated using the method of Bahekar and Kale (2016). This method utilizes the inhibition of auto-oxidation of pyrogallol by superoxide dismutase (SOD) enzyme. 3 mL mixture consisted of 100 µL each of 0.2 mM pyrogallol, 1 mM EDTA, 1 mM DTPA, and 100 µL of serum in air-equilibrated tris-HCl buffer (50 mM; pH 8.2). The reaction mixture prepared in 3 sets includes standard, test, and control. Pyrogallol was added after all other reagents to start the reaction. The initial 10-second period was considered as the induction period of the enzyme. After 10 seconds, a change in absorbance at 420 nm at 10 s intervals was recorded for 4 min. The average change in the absorbance per minute was calculated. One unit of enzyme SOD was defined as the amount of enzyme to cause 50% inhibition of pyrogallol auto-oxidation.

Estimation of reduced glutathione

Reduced glutathione was estimated following the method of Gabriel-Brisibe et al. (2020). The method is based on developing a yellow color when DTNB (Ellman's Reagent) is added to sulphydryl compounds due to a redox reaction between GSH and DTNB. The developed color was reasonably stable for about 10 min, and temperature variation slightly affected the reaction. The absorbance was measured at 412 nm. GSH in red cells is relatively stable, and venous blood samples anticoagulated with ACD maintain GSH levels for up to 3 weeks at 4°C.

Estimation of catalase

Catalase was estimated using the method of Bahekar and Kale (2016) and Gabriel-Brisibe et al. (2020). This method carefully controls the reduction of dichromate in acetic acid to chromate acetate when heated with H_2O_2, ensuring a predictable reaction. The resulting chromic acetate was measured colorimetrically at 570 nm. Catalase (CAT) enables the separation of H_2O_2. Three sets of tubes were prepared and labeled as blank, test (0 s), and test (60 s) and added with the appropriate reagents to each tube. The tubes were boiled for 10 minutes, cooled to room temperature, and the absorbance was read at 570 nm. The analysis used various H_2O_2 concentrations ranging from 10 to 160 µmoles. One unit of catalase (CAT) activity is defined as the amount of enzyme that decomposes 1 µmole of H_2O_2 per minute.

Evaluation of the lipid profile

After the separation of serum from the whole blood, the various parameters of the lipid profile were estimated using standard laboratory procedures: Total Cholesterol (TC) (Stockbridge et al. 1989), Triglycerides (TG) (Annoni et al. 1982), Low-Density Lipoprotein Cholesterol (LDL) and High-Density Lipoprotein Cholesterol (HDL) (Assmann

1979). Serum LDL was calculated using the following formula by Odinga et al. (2020):

$$\text{LDL (mg/dL)} = \frac{TC-HDL-TG}{5}$$

Evaluation of the clinical markers

Blood was collected in 10 mL heparin-coated tubes and centrifuged without delay. Cells were discarded, and plasma was analyzed using the method described by de Winter et al. (1995) and Fiolet et al. (1977):

Myoglobin

The myoglobin assay (Turbiquant myoglobin, Behringwerke) was used with the Behring Turbitimer analyzer for rapid immunoturbidimetric determination of myoglobin concentrations in plasma. This assay is based on polystyrene particles coated with rabbit anti-human myoglobin antibodies, which form agglutinates with myoglobin present in serum or plasma. The agglutination causes an increase in turbidity, which is measured with a photometer. The measurement range was 50 to 650 ng/mL. The upper reference limit was 90 ng/mL. The turnaround time of the assay was 20 minutes.

Troponin T

Troponin T was measured using an ELISA method (Boehringer Mannheim, product 1289055) on an ES300 analyzer (Boehringer Mannheim). The upper reference limit was 0.1 ng/mL, and the linearity range of this determination was 0 to 15 ng/mL. The turnaround time of the assay was 2.5 hours.

Statistical analysis

IBM SPSS Version 25 was used to analyze the data. The data were analyzed using one-way analysis of variance (ANOVA) and presented as mean ± standard deviation, and Turkey's post hoc test was used for multiple comparisons. $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results in Table 2 indicated differences in the percentage body weight gain of hyperlipidemic rats treated with different *K. pinnata* extract concentrations. The 400 mg/kg BW treatment had the lowest body weight gain, similar to normal control. The body weight gain of the rats, as shown in their final body weight, could be attributed to their food and water intake *ad libitum* throughout the experimental period with a high-fat diet to induce hyperlipidemia. Odinga et al. (2023) reported that feed with a high content of nutrients and calories increases body weight.

Table 2. % Mean body weight difference of experimental rats

Group	Initial weight (g)	Final body weight (g)	% mean body weight difference
Normal control	159.00±21.49	178.67±8.62	12.37
Negative control	133.67±2.67	171.67±17.17	28.43
Positive control	160.50±2.59	194.00±22.69	19.38
200 mg/kg *K. Pinnata*	153.67±3.98	196.50±31.14	27.87
400 mg/kg *K. pinnata*	146.17±5.49	166.33±16.59	13.79

Note: Values are mean (M) ± Standard Deviation (SD)

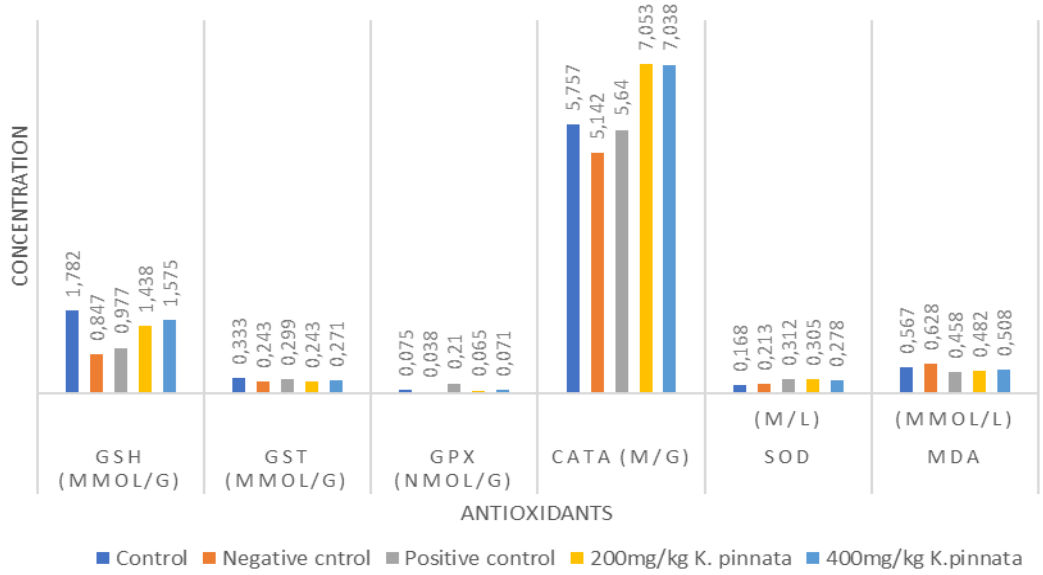

Figure 1. Effect of aqueous extract of *Kalanchoe pinnata* on the antioxidant status of experimental rat model

Table 3. Lipid profile of hyperlipidemic rats treated with aqueous extract of *Kalanchoe pinnata*

Groups	TC (mmol/L)	TG (mmol/L)	HDLC (mmol/L)	LDLC (mmol/L)
Control	3.067±0.493a	1.397±0.228a	1.280±0.232a	2.458±0.391a
Negative control	3.167±0.441a	1.502±0.224a	1.310±0.279a	2.362±0.289ab
Positive control	2.667±0.829a	1.303±0.296a	1.428±0.213ac	1.930±0.839c
200mg/kg *K. pinnata*	2.783±0.412a	1.312±0.227a	1.455±0.283d	1.937±0.383d
400mg/kg *K. pinnata*	2.700±0.693a	1.203±0.355ae	1.487±0.322de	1.935±0.798e

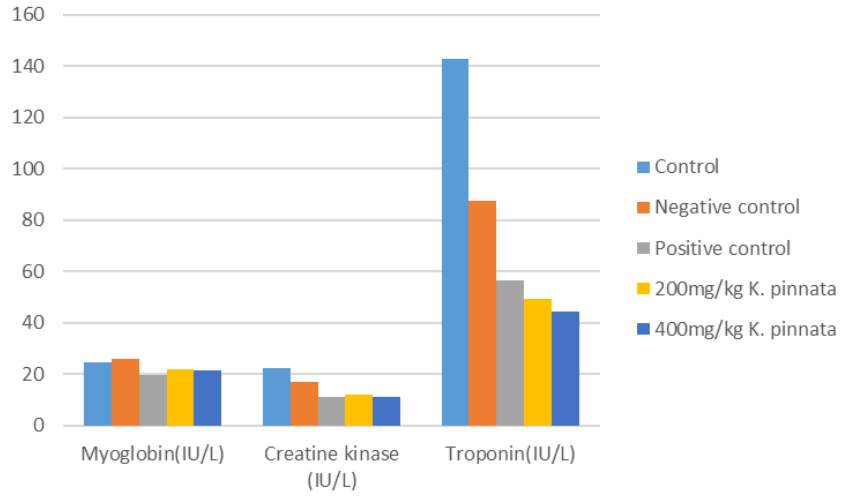

Figure 2. Effect of aqueous extract of *Kalanchoe pinnata* on the myoglobin, creatine kinase, and troponin serum levels of experimental rat model

Figure 1 represents the antioxidant status of experimental hyperlipidemic rats administered with the aqueous extract of *K. pinnata*. The result revealed that the administration of *K. pinnata* extract increased the serum GSH, GST, catalase, and SOD level of the rats. However, the serum MDA and GPx decreased. These findings indicated that the extract contained a significant amount of antioxidants, as evidenced by the increased concentrations of the antioxidant markers GSH, GST, GPx, catalase, and SOD in the serum of rats administered 200 mg/kg and 400 mg/kg of *K. pinnata.* These antioxidant parameters in the treated groups were higher than those in the negative control group, which was not administered with the extract of *K. pinnata* after the inducement of hyperlipidemia. Ramon et al. (2023) reported that *K. pinnata* contained quercetin, kaempferol, apigenin, ECGC, and avicularin, which have antioxidant activity. The findings suggest that the increase in antioxidant markers following the administration of the aqueous extract of *K. pinnata* may be related to its phytochemical contents. It also indicates that the extract of *K. pinnata* has therapeutic potential against diabetes and inflammation.

Glutathione (GSH) is crucial in cellular processes and redox homeostasis. Any deficiency or imbalance in the GSH/GSSG ratio increases cell susceptibility to oxidative stress, inflammation, and tumor development. Conversely, increased GSH levels increase antioxidant capacity and resistance to oxidative stress, which is observed in many types of tumors. The addition of exogenous GSH inhibited the inflammatory response by regulating Reactive Oxygen Species (ROS). However, the role of endogenous GSH in fine-tuning the innate immune response and thus modulating inflammation is also highly significant. GSH acts as an antioxidant, scavenging ROS during oxidative stress, and a signaling molecule that regulates protein function through thiol-disulfide exchange reactions, such as protein glutathionylation. Previous studies showed that GSH can regulate the activity of various oncogenes (e.g., p53, HIF-1, c-jun) through these mechanisms (Kennedy et al. 2020).

Pahwa et al. (2017) reported that increased GST activity in cardiovascular disease patients, especially those with type 2 diabetes, suggests a protective mechanism against increased oxidative stress. Glutathione S-Transferase (GST) is an important enzyme in detoxification and helps lower oxidative stress. This enzyme might be induced under oxidative stress conditions as a protective mechanism.

GPx-1 is a crucial antioxidant enzyme that prevents the harmful accumulation of intracellular hydrogen peroxide. It is present in all cells and is found in cytosolic, mitochondrial, and, in some cells, peroxisomal compartments. It is more effective than catalase at removing intracellular peroxides under many physiological conditions (Savas et al. 2006).

Nandi et al. (2019) noted that catalase is one of the crucial antioxidant enzymes that mitigates oxidative stress by destroying cellular hydrogen peroxide to produce water

and oxygen. Its deficiency or malfunction is related to the pathogenesis of many age-associated degenerative diseases like diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia.

SOD is an enzymatic antioxidant that catalyzes the conversion of O_2 to H_2O_2 and helps maintain the redox balance by diffusing the superoxide. Therapeutically, increasing the levels of SOD could be a strategy in oxidative stress-induced pathology (Xue et al. 2021). MDA has been reported as a polyunsaturated fatty acid peroxidation product (Gaweł et al. 2004); thus, it implies the pathogenesis of various disease conditions. Therefore, the MDA level was expected to be decreased by *K. pinnata* administration.

The antioxidant activity in this study may be due to the combined action of the bioflavonoids in *K. pinnata*. Quercetin, a natural flavonoid detected in *K. pinnata*, is therapeutic against type II diabetes by acting as an anti-inflammatory and antioxidant. Hence, *K.pinnata is* a promising source of natural antioxidants.

Table 3 revealed a decrease in the serum TC concentration and Low-Density Lipoprotein Cholesterol (LDLC) of the experimental hyperlipidemic rats administered with 200 mg/kg and 400 mg/kg of *K. pinnata.* The serum HDLC increased in the groups treated with 200 and 400 mg/kg aqueous extracts of *K. pinnata*.

HFD has been used to induce hyperlipidemia in rat models (Pande and Dubey 2009) and cause an elevation in the TC and LDL Cholesterol levels in the serum (Sampathkumar et al. 2011; Odinga et al. 2020). High serum levels of LDL Cholesterol could predispose to most cardiovascular diseases, such as atherosclerosis (Ahmad et al. 2018)

Table 2 showed a decrease in TC, TG, and LDLC concentration in the groups treated with 200 mg/kg and 400 mg/kg aqueous extracts of *K. pinnata* compared to the normal and negative groups; however, the levels of HDLC increased. However, the 400 mg/kg of K. pinnata administration caused the most significant decrease in TC, TG, and LDLC levels. Ahmad et al. (2018) reported that plant extract lowers TC levels by increasing bile acid excretion and preventing reabsorption from the small intestine by disrupting bile acid's micelle formation. Plants' ability to lower TC may be due to the phytochemical composition of plants (Rabizadeh et al. 2022). The increased excretion of bile acid and cholesterol activates cholesterol 7α-hydroxylase and enhances the conversion of liver cholesterol to bile acid, thus reducing cholesterol.

Triglyceride levels in the serum of the experimental rats were significantly reduced after 21 days of treatment with 400 mg/kg of *K. pinnata* (Table 2). Elevated serum TG levels are indicative of pathological conditions related to arterial hardening, which increases the risk of stroke, heart attacks, and heart disease (Jin et al. 2023). Elevated TG levels could arise from complications associated with a high-fat diet, such as obesity (Jin et al. 2023). The reduction in the TG level in the group treated with 200 and 400 mg/kg *K. pinnata* could be attributed to the presence of glycosides in *K. pinnata* (Pavani et al. 2024) that could enhance the lipase enzyme activity in the liver, thereby resulting in the catabolism of lipids. Singh et al. (2013) and Akbarzadeh et al. (2015) also reported that a decrease in TG could also be due to the inhibition of dietary lipid absorption in the intestine by reducing micellar solubilization of cholesterols and by increasing the excretion of TG through feces.

The present study showed increased serum HDLC levels in hyperlipidemic rats treated with *K. pinnata* extract at 200 mg/kg and 400 mg/kg BW. High HDL levels are associated with a lower risk of heart disease. Cho and Jung (2021) showed that the higher the HDL-C level, the better in lowering mortality caused by cardiovascular disease and myocardial infarction risk. Odinga et al. (2020) reported that HDLC helps remove cholesterol from the bloodstream and returns to the liver, where it is catabolized and excreted from the body. It implies that an increase in HDLC serum levels is a good indication. HDLC is commonly known as the good cholesterol.

The LDLC levels in the groups treated with 200 mg/kg and 400 mg/kg BW of *K. pinnata* were lower than the normal and negative control groups. Serum LDLC, also known as bad cholesterol, might be caused by saturated fats and cholesterol in high-fat feeds. LDLC could cause fatty deposits in blood vessels, blocking blood flow in the arteries (Rafieian-Kopaei et al. 2014). The fatty deposits have the potency to form clots, leading to myocardial infarction and stroke (Tanka-Salamon et al. 2016). Ivanova et al. (2021) reported that a plant-based diet effectively lowers LDL cholesterol. The decreased LDLC in the experimental rats treated with *K. pinnata* extract could be due to bioactive compounds with anti-inflammatory and hypolipidemic activities (Odinga et al. 2016). Odinga et al. (2020), in their study on the Antihyperlipidemic effects of *Ricinodendron heudelotii*, reported that LDLC does not facilitate the removal of cholesterol from the body, unlike HDLC. Instead, it deposits cholesterol onto the walls of blood vessels; therefore, elevated levels of LDLC can lead to the accumulation of cholesterol and triglycerides along critical blood vessels. In the long term, it could lead to cardiovascular diseases. Additionally, Zawirska-Wojtasiak et al. (2019) reported the therapeutic use of the leaves of *K. pinnata* for antimicrobial, anti-inflammatory, and antiseptic activities. They also reported that *K. pinnata* is vitamin C-rich.

Myoglobin, CK/CK-MB, and troponin serum levels are crucial because they diagnose Acute Myocardial Infarction (AMI) during the onset of symptoms and emergencies (de Winter et al. 1995). These levels can be affected by various risk factors (Odum and Young 2018). Chiu et al. (1999) suggested combining myoglobin, CK-MB, and troponin parameters could be valuable information in managing Acute Myocardial Infarction (AMI). Figure 2 shows elevated serum myoglobin in the hyperlipidemic rats without any other treatment or negative control. Duan et al. (2018) and Wali et al. (2020) reported that a high-fat diet might affect body metabolism, heart, and muscles. Woo et al. (1995) stated the importance of determining myoglobin level in diagnosing AMI. The experimental rat groups administered standard drugs and *K. pinnata* at various

concentrations had lower serum myoglobin than the control and negative control groups. Detecting cardiac markers such as troponin, myoglobin, and Heart-type Fatty Acid-Binding Protein (H-FABP) could have higher sensitivity and specificity in diagnosing AMI than any single detection. It can provide better data supporting the AMI diagnosis (Sun et al. 2023). Sax et al. (1997) concluded that the ratio of CK-MBm to CK levels reflects, to some extent, the severity of coronary disease and that pre-infarction beta-blockade may lead to lower CK-MB levels.

In conclusion, the administration of *K. pinnata* aqueous extract to induced hyperlipidemic rats reduced the body weight gain, decreased serum concentrations of LDLC, TG, and TC, and increased HDLC serum concentration. It confirms the hypolipidemic activity of *K. pinnata.* The administration of 400 mg/kg body weight of *K.pinnata* aqueous extract had the best results. With the increasing prevalence of hyperlipidemia, primarily driven by risk factors such as dietary habits, *K. pinnata* appears to be a promising natural remedy. This research indicates that *K. pinnata* could serve as a source of natural medicine, and further studies should investigate its potential as a nutritional supplement.

It is recommended that *K. pinnata* be used as an antioxidant and antihyperlipidemic source. This study recommends further elucidation of the effect of the bioactive compounds in *K. pinnata* on health and their possible dose limits.

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