



Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on selected indices of cardiovascular diseases in mouse

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Abstract

Some anticancer pregnane glycosides, e.g., 3-O-[6-deoxy-3-O-methyl- β -d-allopyranosyl-(1 $\rightarrow$ 4)- β -d-canaropyranosyl]-17 β -marsdenin (3DMACM), have been isolated from *Gongronema latifolium* leaves which have not been evaluated for their effects on the cardiovascular system. Thus, this study was carried out to evaluate the effects of 3DMACM on selected indices of cardiovascular diseases in albino mice. Forty mice were randomly divided into four groups (ten mice each). Group A mice were orally administered 5% DMSO while mice in groups B, C, and D were orally administered 0.25, 0.5, and 1 mg/kg body weight of the compound for 14 days, after which various indices of cardiovascular diseases were determined. The results revealed that the total cholesterol, triacylglycerol, very low density lipoprotein-, and high-density lipoprotein-cholesterol concentrations in the plasma and heart Na^+ - K^+ -ATPase activity were significantly reduced ($p < 0.05$) at the doses of 0.25 and 1 mg/kg body weight of the compound compared to controls. Also, atherogenic index, plasma LDH activity, and heart Mg^{2+} -ATPase activity of the mice were significantly ($p < 0.05$) reduced at all doses of the compound compared to controls. Heart and plasma creatine kinase activities and heart Ca^{2+} - Mg^{2+} -ATPase activity of mice were significantly ($p < 0.05$) reduced at doses higher than 0.25 mg/kg body weight of the compound compared to controls. AST activities in the heart and plasma of mice were significantly increased ($p < 0.05$) at doses of 0.25 and 1 mg/kg body weight compared to controls. The results suggest that 3DMACM may not predispose subjects to atherosclerosis but may adversely affect cardiac muscle contraction/relaxation, especially at higher doses.

Keywords Pregnane glycoside · Cardiovascular system · Toxicity · Plant · Muscle contractility

Introduction

Natural products have over the years been a dependable and inexhaustible source of remedies for the treatment of various diseases, especially chronic diseases, such as cancer, diabetes,

malaria, and heart diseases (Newman and Cragg 2012; Miao et al. 2016). In African traditional system of medicine, plant-based raw materials, such as the roots, stem bark and leaves, are predominantly employed for the treatment of various diseases. However, some of these plants have been reported to exert toxic effects on subjects (Dzoyem et al. 2014). This has led to the isolation of pure compounds responsible for the pharmacological activities of such plants in order to reduce the toxicity of the crude extracts. Unfortunately, some of these compounds have also been reported to exert toxic effects, despite their pharmacological activities, which have rendered them clinically useless.

A plant that is very useful in Nigerian indigenous medicine from which various phytochemicals with diverse pharmacological activities have been isolated is *Gongronema latifolium*. It is a type of vegetable consumed regularly in the southern part of Nigeria and used in folk medicine for the treatment of various diseases such as malaria, anorexia, constipation, nausea, hypertension,

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diabetes, stomach ache, cough, dyspepsia, and dysentery (Morebise 1998; Morebise et al. 2006; Mohammed et al. 2014). Phytochemicals such as B-sistosterol, lupeynyl esters, pregnane ester, glycosides, essential oils, and saponins have been reported to be present in different parts of this herb (Schneider et al. 1993; Morebise 1998; Morebise et al. 2006). Aside the reported ethnopharmacological activities of the plant, the effects on cardiovascular indices, especially in diseased states, in rats have been reported by several authors (Edet et al. 2009, 2011a, b; Okpala et al. 2014). Akinnuga et al. (2011) reported the significant decrease in selected red and white blood cell indices and the platelet count of rats caused by the ethanolic leaf extract of the plant after fourteen days of administration. However, Beshel et al. (2019) has reported the cardioprotective effects of the ethanolic leaf extract in normal rats with no pathological conditions.

Of recent one pregnane and three pregnane glycosides isolated from the leaf of *Gongronema latifolium* were found to exhibit antileukemic activities (Gyebi et al. 2018). One of these pregnane glycosides is 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin (3DMACM) (Fig. 1). However, there is paucity of information on the effects of this compound on the cardiovascular system, since the ethanolic leaf extract of *G. latifolium* has been reported to have cardioprotective effects (Beshel et al. 2019). This study was, therefore, carried out to evaluate the effects of 3DMACM using selected indices of cardiovascular diseases in mouse.

Materials and methods

Chemicals

Assay kits for total- and HDL-cholesterol and triacylglycerol concentrations were obtained from Randox Laboratories

Ltd., Co-Antrim, UK. Other chemicals used were of analytical grade and prepared in all glass distilled water. The compound 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 4)- β -D-canaropyranosyl]-17 β -marsdenin was isolated from our previous study (Gyebi et al. 2018).

Experimental animals

A total of forty (40) albino mice were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed under laboratory conditions of 12 h light–dark cycle and room temperature of 25 ± 2 °C. The animals were allowed access to standard laboratory feeds (Top Feeds, Ilorin, Nigeria) and clean water ad libitum. The animals were handled according to the guidelines stipulated for animal care (Clark et al. 1997; Garber 2011).

Animal grouping and drug administration

The forty albino female mice were randomly divided into four groups (A, B, C, and D) of 10 mice each. Daily administration of 3DMACM was done orally for fourteen (14) days. Group A (control) mice were orally administered 5% DMSO, while groups B, C, and D mice were administered 0.25, 0.5, and 1 mg/kg body weight of the compound respectively. The selected doses were based on the available amount of compound isolated from the chromatographic fractionation of the crude leaf extract of the plant.

Sample collection and preparation

After 24 h of the administration of the last set of doses of the compound, the animals were sacrificed by slightly anaesthetizing them with diethyl ether. Venous blood was collected into EDTA bottles for hematological analysis and lithium

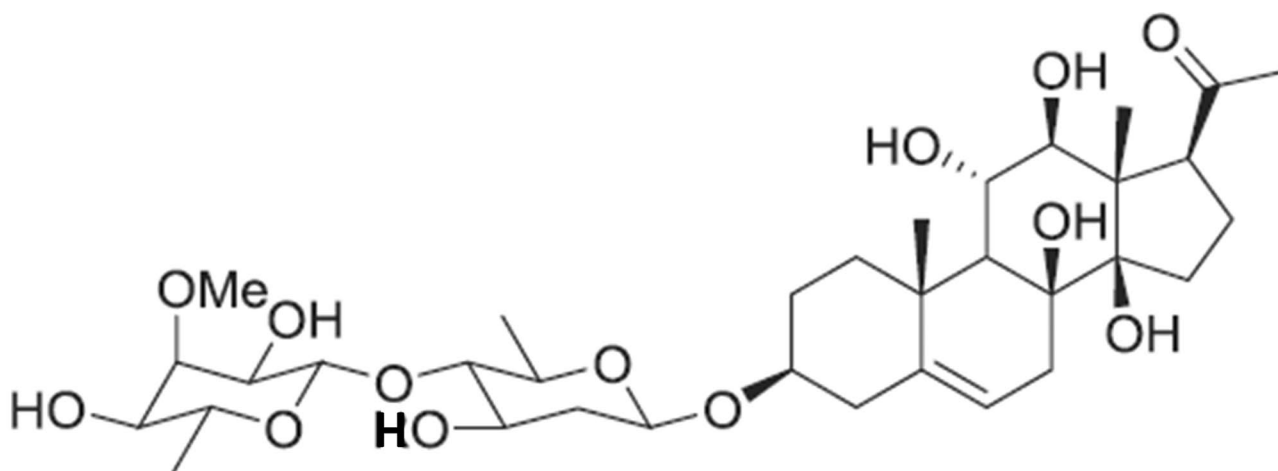


Fig. 1 Structure of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin

heparin bottles for preparation of plasma. The blood was centrifuged at 8000 rpm for 3 min. The plasma of the blood samples was pipetted out and stored at -20°C until needed for analysis. The heart was isolated, cleaned of blood, and homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored at -20°C overnight to ensure maximum release of the enzymes in the heart homogenate (Akanji et al. 1993).

Biochemical assays

Protein concentrations of the heart homogenates and plasma were determined by the method of Gornall et al. (1949). The plasma total cholesterol concentration was determined using the enzymatic saponification procedure described by Allain et al. (1974). Plasma triacylglycerol concentration was determined using the method described by Tietz (1990). The method described by Bachorik et al. (1980) was used to determine plasma HDL-cholesterol concentration. Plasma LDL-cholesterol and VLDL concentrations were calculated using the formulae described by Friedewald et al. (1972). The atherogenic index was calculated using the formula, $\text{LDL-C}/\text{HDL-C}$ Nicholls et al. (2007). The method described by Weisshaar et al. (1975) was used for the determination of lactate dehydrogenase activity. The method described by Reitman and Frankel (1957) was used for the determination of aspartate aminotransferase activity. Mg^{2+} -ATPase activity was determined using the method described by Ronner et al. (1977) as modified by Fleschner and Kraus-Friedmann (1986). Na^{+} , K^{+} ATPase, and Ca^{2+} - Mg^{2+} ATPase activities were determined by the method of Ronner et al. (1977) as modified by Bewaji et al. (1985). The method described by Witt and Trendelenburg (1982) was used for determining creatine kinase-MB activity.

Haematological parameters

The red blood cell count, packed cell volume, reticulocyte count, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, haemoglobin concentration, white blood cell count, percentage neutrophils, percentage lymphocytes, and platelet count were determined using an automated haematological analyser SYSMEX KX21 (SYSMEX Corp, Japan).

Statistical analysis

Data are expressed as the means $\pm$ SEM of 5 determinations. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Duncan multiple range test as post hoc test using GraphPad Prism software (GraphPad Software, California, USA). Differences were considered statistically significant at $p < 0.05$.

Results

Heart-body weight ratio of mice

3DMACM caused significant reduction ($p < 0.05$) in the heart-body weight ratio of mice administered 1 mg/kg body weight with no significant alteration ($p > 0.05$) at other doses compared to control (Fig. 2).

Serum lipid profile

3DMACM significantly reduced ($p < 0.05$) plasma total cholesterol, HDL-cholesterol, VLDL and triacylglycerol concentrations of mice administered 0.25 and 1 mg/kg body weight of the compound with no significant change ($p > 0.05$) at 0.5 mg/kg body weight of the compound compared to controls (Figs. 3, 4, 5, and 6). There was significant reduction ($p < 0.05$) in atherogenic index of mice at all doses of the compound compared to control (Fig. 7).

Enzymes

There was significant reduction ($p < 0.05$) in heart Mg^{2+} ATPase and Na^{+} , K^{+} -ATPase activities of mice at all doses of 3DMACM administered compared to controls (Figs. 8 and 9). There was significant reduction ($p < 0.05$) in heart Ca^{2+} - Mg^{2+} ATPase activity of mice administered 0.5 and 1 mg/kg body weight of the compound with no significant change ($p > 0.05$) at 0.25 mg/kg body weight compared to control (Fig. 10). There was significant increase ($p < 0.05$) in AST activities in the plasma and heart of mice administered 0.25 and 1 mg/kg body weight of the compound with significant decrease ($p < 0.05$) in plasma AST activity and no significant change ($p > 0.05$) in heart AST activity in mice at the dose of 0.5 mg/kg body weight of the compound compared to controls (Fig. 11). There was significant reduction ($p < 0.05$) in creatine kinase activity in the heart and plasma of mice administered 0.5 and 1 mg/kg body weight of the compound with no significant change ($p > 0.05$) at 0.25 mg/kg body weight compared to controls (Fig. 12). There was significant reduction ($p < 0.05$) in lactate dehydrogenase activity in the plasma of mice at all doses of the compound compared to controls (Fig. 13).

Haematological parameters

The platelet count and percentage lymphocyte were significantly reduced ($p < 0.05$) at all doses of 3DMACM compared to controls (Table 1). The red blood cell count (RBC) was significantly reduced ($p < 0.05$) at doses higher than 0.25 mg/kg body weight of the compound, while the packed cell volume was only significantly reduced ($p < 0.05$) at the highest dose

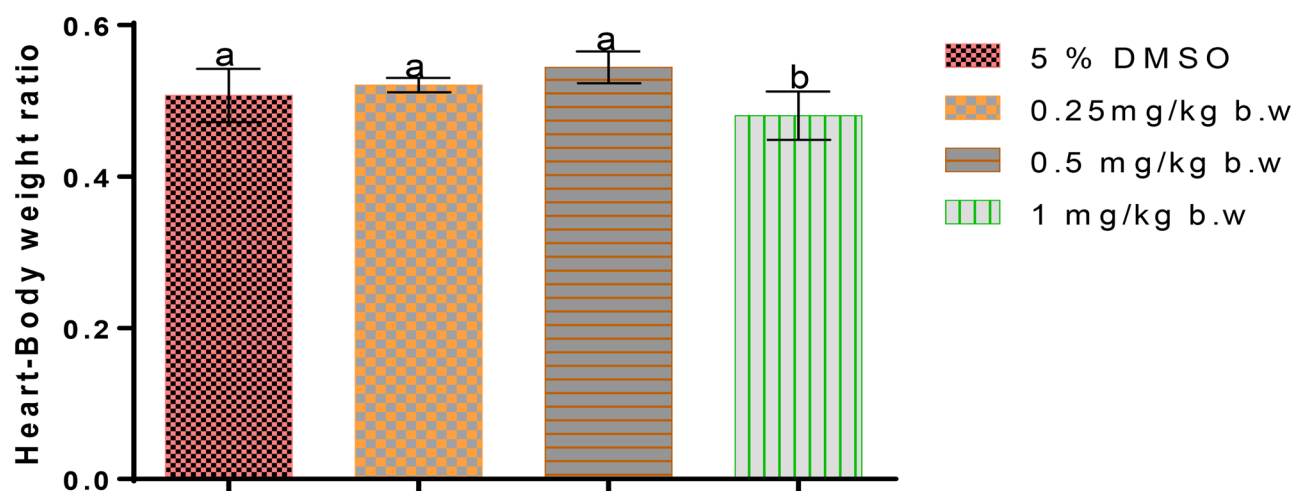


Fig. 2 Effects of 3-O-[6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl]-17β-marsdenin on heart-body weight ratios of albino mice. Values are expressed as means of five replicates ± SEM.

Bars with different superscripts are significantly different ($p < 0.05$) from each other

compared to controls (Table 1). The MCV was significantly increased ($p < 0.05$) at 1 mg/kg body weight of the compound, while the MCH, MCHC, and the total white blood cell count were not significantly ($p > 0.05$) altered at all doses compared to controls (Table 1).

Discussion

Organ-body weight ratio

Organ-body weight ratio is normally investigated to determine whether the size of the organ has changed in relation to the

weight of the whole animal. High organ/body weight ratio suggests inflammation, while low organ/body weight ratio is associated with constriction resulting from necrosis (Moore and Dalley 2018). The administration of 1 mg/kg body weight of the compound caused significant reduction ($p < 0.05$) in organ/body weight ratio compared to the control, which may be due to organ constriction resulting from necrosis (cell death).

Cardiovascular disease indices

Alteration in the concentrations of major lipids such as total cholesterol, HDL-C, LDL-C, and triacylglycerols can

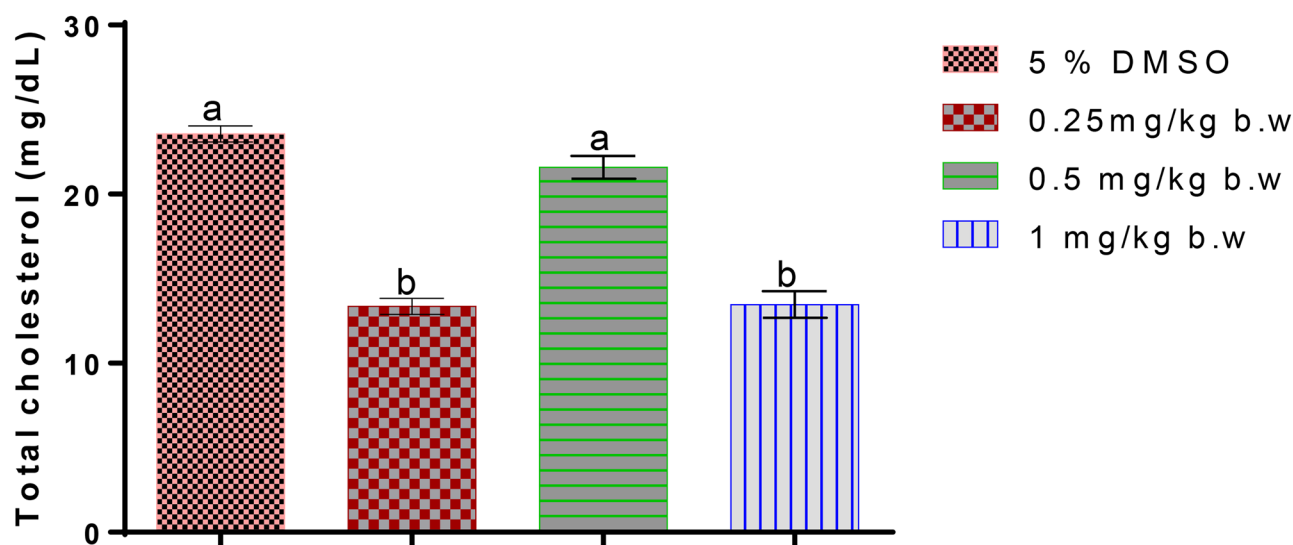


Fig. 3 Effects of 3-O-[6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl]-17β-marsdenin on total cholesterol concentration in the plasma of mice. Values are expressed as means of five

replicates ± SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

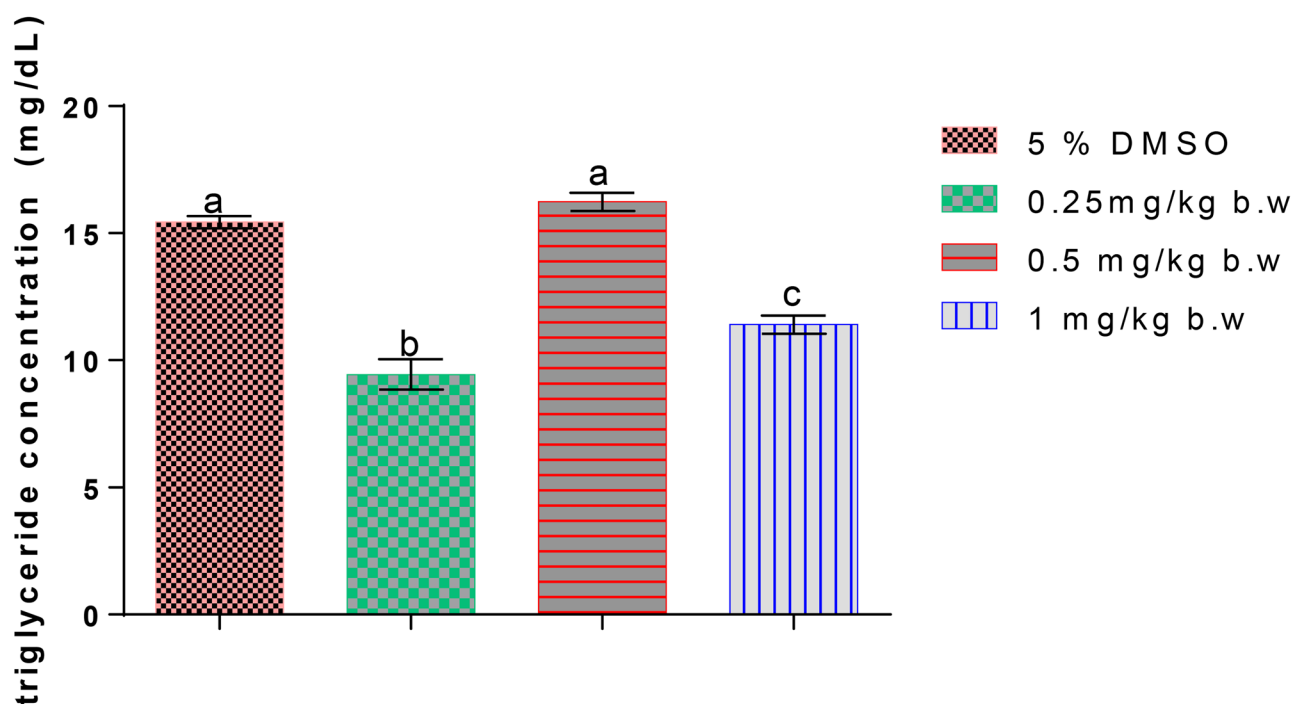


Fig. 4 Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on triacylglycerol concentration in the plasma of mice. Values are expressed as means of five

replicates $\pm$ SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

give useful information on lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Deans and Dominiczak 2008; Stamler et al. 1986). Triacylglycerols and VLDL have clinical values in assessing coronary heart diseases (Deans and Dominiczak 2008), though not independent markers. High plasma cholesterol concentration is an important risk factor to be considered for cardiovascular diseases (Abolaji et al. 2007). LDL is the primary carrier of cholesterol to peripheral tissues. LDL-C (often designated bad cholesterol) in the plasma is linked with increased deposition of cholesterol in the arterial walls (Olukanni et al. 2013; Vander et al. 1998). As a result, it forms plaque in the arteries, thereby causing atherosclerosis. The administration of 3DMACM at doses of 0.25 and 1 mg/kg body weight led to reduction in the concentrations of total cholesterol, triacylglycerol, and VLDL in the plasma compared to controls. This suggests that the compound at these doses may impair the synthesis of cholesterol, triacylglycerol, and VLDL in the liver, which may not predispose subjects to cardiovascular diseases. This is corroborated by the observed reduction in plasma LDL-C concentration and atherogenic index at all doses of the compound compared to controls. This suggests that 3DMACM was one of the compounds responsible for the reduction in serum LDL-C concentration caused by ethanolic extract

of *G. latifolium* leaf (Beshel et al. 2019). However, the concentration of HDL-C (which is known to have anti-atherogenic property) was significantly reduced ($p < 0.05$) at 0.25 and 1 mg/kg body weight of 3DMACM, suggesting that the role of the HDL in mopping up cholesterol back to the liver may be adversely affected. This suggests that 3DMACM was not the compound responsible for the increase in serum HDL-C concentration caused by ethanolic extract of *G. latifolium* leaf (Beshel et al. 2019).

Enzymes are sensitive tools used for the diagnosis of damaged tissue long before the manifestation of histological changes (Malomo 2000). Therefore, the measurement of the activities of “marker” enzymes in tissues and body fluids can be used in assessing the degree of assault and toxicity of a chemical compound on organs/tissues (Malomo 2000; Yakubu et al. 2007). These non-plasma specific enzymes (transferases, phosphatases, and dehydrogenases) get into the blood through plasma membrane leakage of a damaged tissue (Wright et al. 1974). In this study, increase in AST activities in both the plasma and heart was observed in the groups administered 0.5 and 1 mg/kg body weight of the compound compared to controls, which may suggest plasma membrane leakage of damaged tissues or activation of the enzyme in situ at those doses.

Na^+/K^+ -ATPase appears to be involved in a number of clinical disorders including cardiovascular diseases and

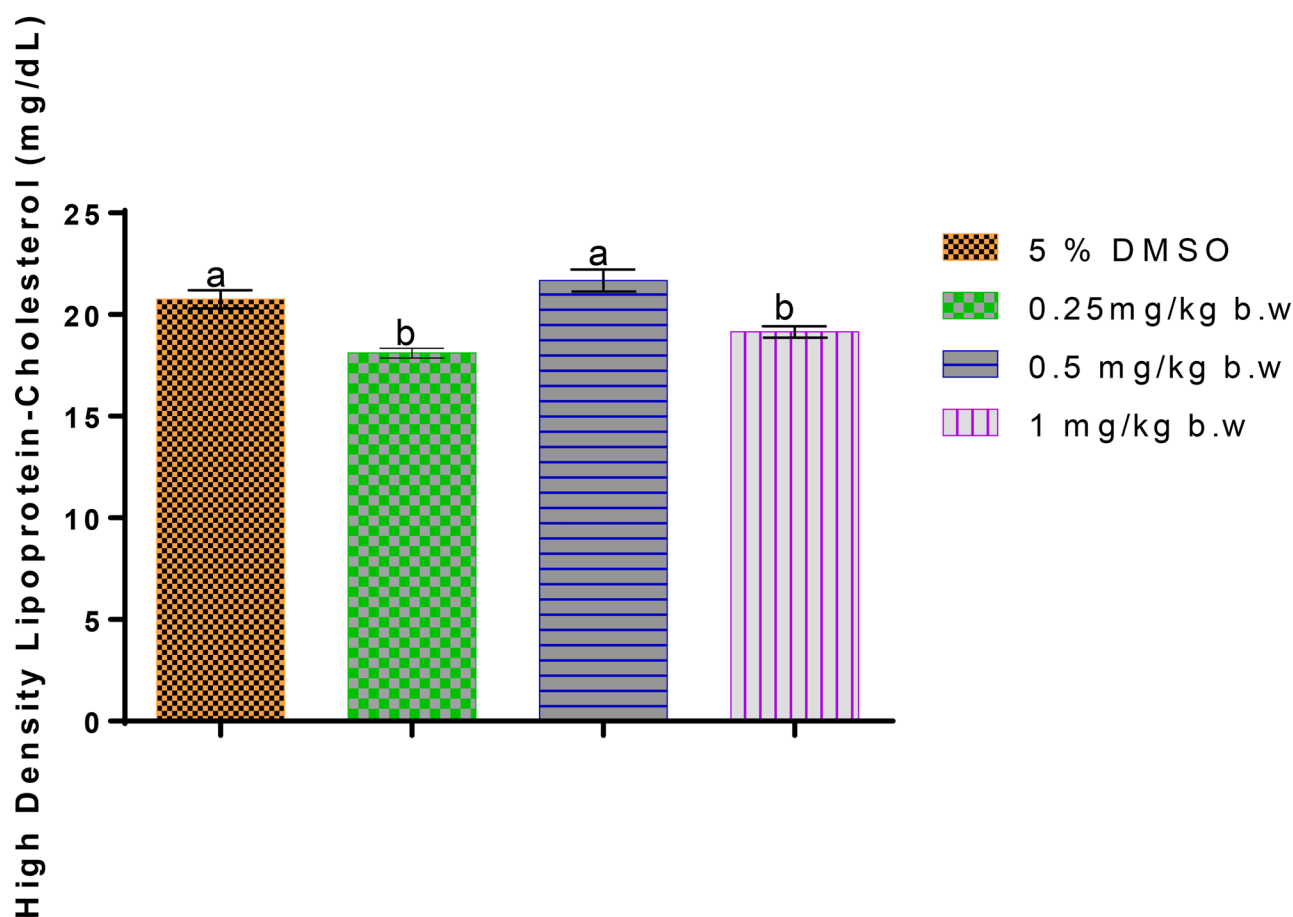


Fig. 5 Effects of 3-O-[6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl]-17β-marsdenin on HDL-C concentration in the plasma of albino mice. Values are expressed as means of five rep-

licates ± SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

hypertension, salt balance, renal diseases, diabetes, and other metabolic diseases, as well as neurological disorders (Bagrov and Shapiro 2008; Bagrov et al. 2009). Moreover, the alteration of signalling receptor function is also seen in hypertension, cardiac hypertrophy, ischemia/reperfusion injury, cancer, and tissue fibrosis. Na^+/K^+ -ATPase helps maintain resting potential, facilitate transport, and regulate cellular volume. It also functions as a signal transducer/integrator to regulate MAPK pathway, ROS, as well as intracellular calcium (Desfrere et al. 2009). In most animal cells, the Na^+/K^+ -ATPase is responsible for about one-fifth of the energy expenditure of the cell (Erecinska and Silver 1989). The expression of Na^+/K^+ -ATPase is upregulated by cAMP (Ahmad and Medford 1995). Compounds which cause increase in cAMP upregulate Na^+/K^+ -ATPase expression. In contrast, compounds causing decrease in cAMP concentration downregulate the expression of Na^+/K^+ -ATPase (Burnier 2007). The administration of 3DMACM at doses of 0.25 and 1 mg/kg body weight led to reduction in Na^+/K^+ -ATPase activity compared to control. This suggests that the compound

at these concentrations may inhibit Na^+/K^+ -ATPase in situ or suppress its expression. A decrease in Na^+/K^+ -ATPase activity may (i) disrupt the cell membrane potential; (ii) adversely affect membrane transport proteins which play critical roles in endocytosis, endocytoglycosis, and transport of other nutrients using sodium gradient; (iii) lead to cardiac myocyte inflammation, which could eventually lead to myocytolysis; (iv) lead to downregulation of the expression of effector proteins of the mitogen-activated protein kinase (MAPK) signalling pathway; and (v) adversely affect both energy metabolism and transmission of the action potential along the T-tubules of the cardiac muscle to initiate the release of calcium ions from the sarcoplasmic reticulum (Bagrov and Shapiro 2008; Bagrov et al. 2009; Berry et al. 2007; Cui and Xie 2017; Liu and Xie 2010).

Plasma membrane Ca^{2+} ATPase (PMCA) is a transport protein in the plasma membrane of cells that serves to remove calcium (Ca^{2+}) from the cell and has been classified as a member of the P-type transport ATPase family, due to the covalent intermediate state that is formed upon

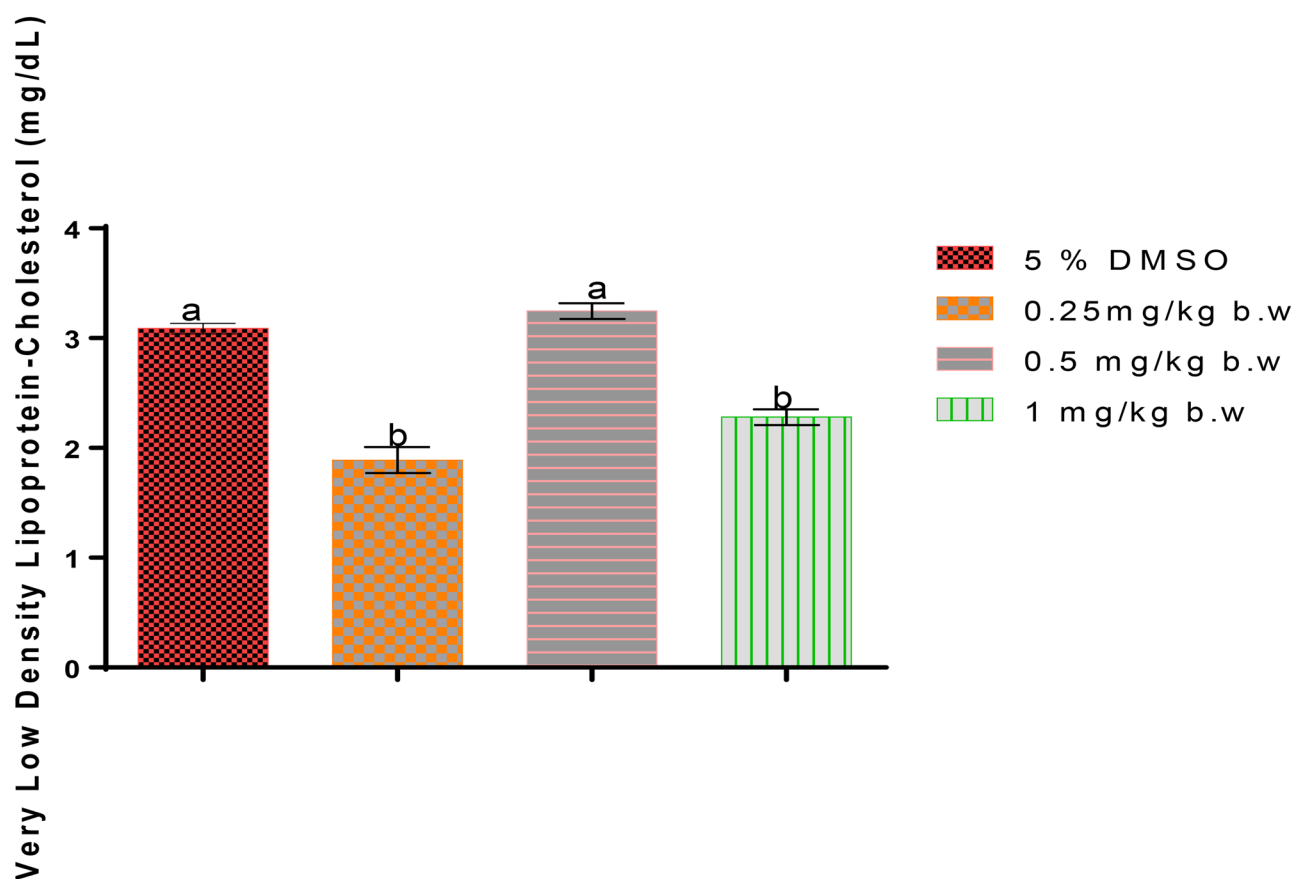


Fig. 6 Effects of 3-O-[6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl]-17β-marsdenin on VLDL concentration in the plasma of albino mice. Values are expressed as means of five repli-

cates ± SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

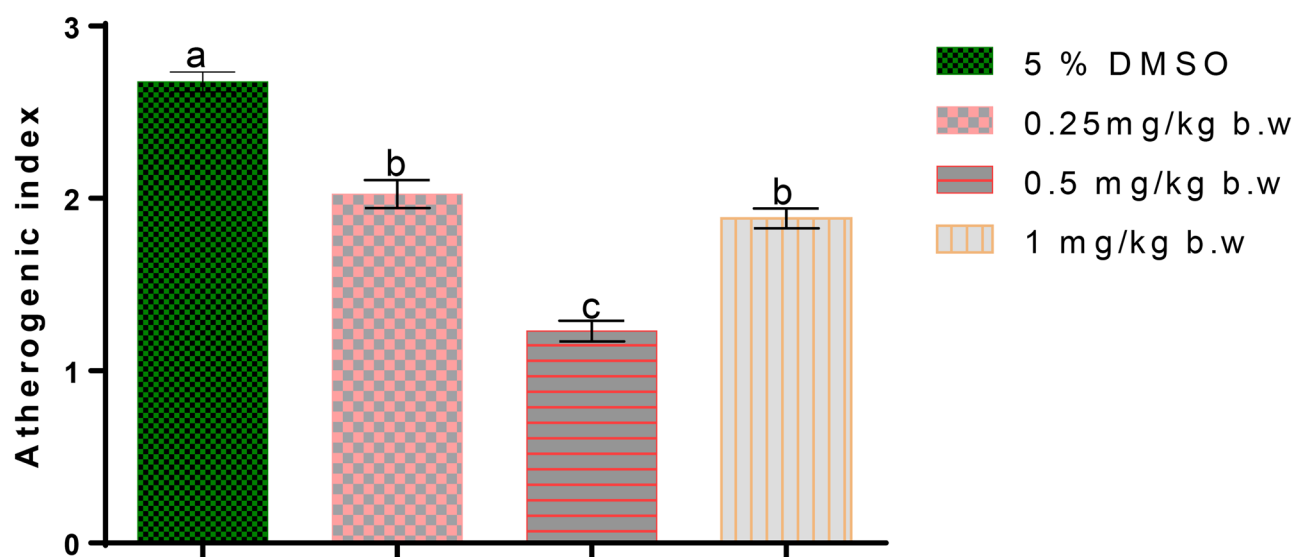


Fig. 7 Effects of 3-O-[6-deoxy-3-O-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl]-17β-marsdenin on atherogenic index of mice. Values are expressed as means of five replicates ± SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

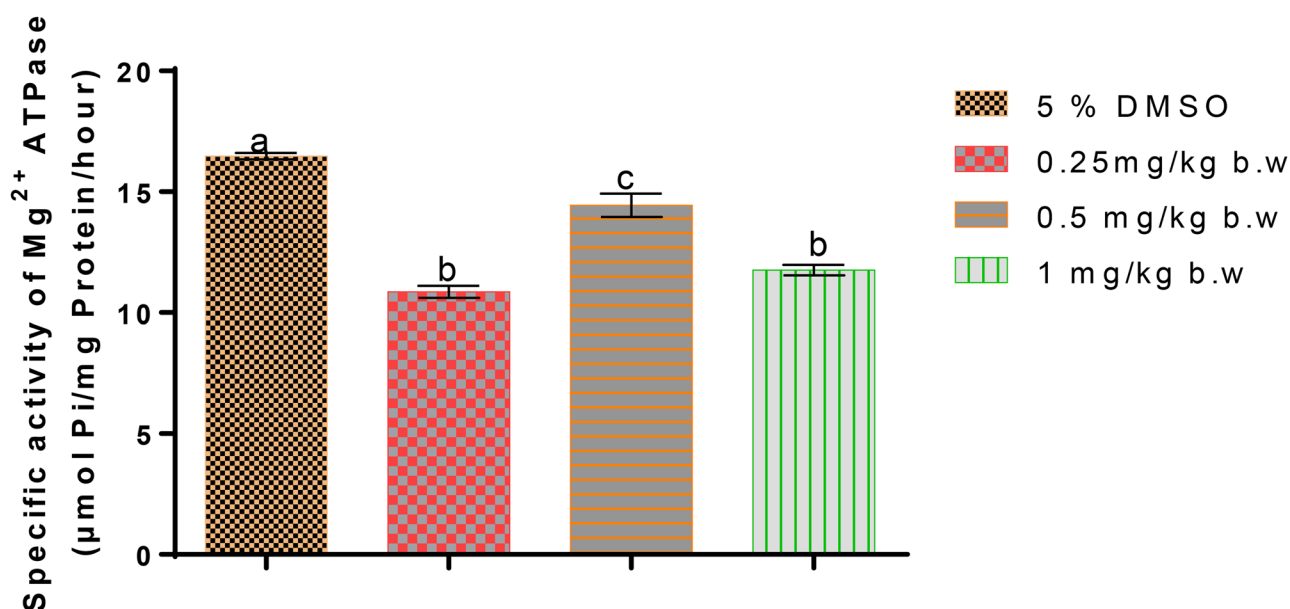


Fig. 8 Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on heart Mg^{2+} -ATPase activity of mice. Values are expressed as means of five replicates $\pm$ SEM. Bars

with different superscripts are significantly ($p < 0.05$) different from each other

phosphorylation of a highly conserved aspartate residue during its catalytic cycle. It is vital for regulating the amount of Ca^{2+} within cells (Jensen et al. 2004). The Ca^{2+} ATPase of sarcoplasmic reticulum has a prominent role in excitation/

contraction coupling of cardiac muscle, as it induces relaxation by sequestering Ca^{2+} from the cytoplasm into intracellular stores delimited by the sarcoplasmic reticulum membranes. The stored Ca^{2+} is in turn released into the

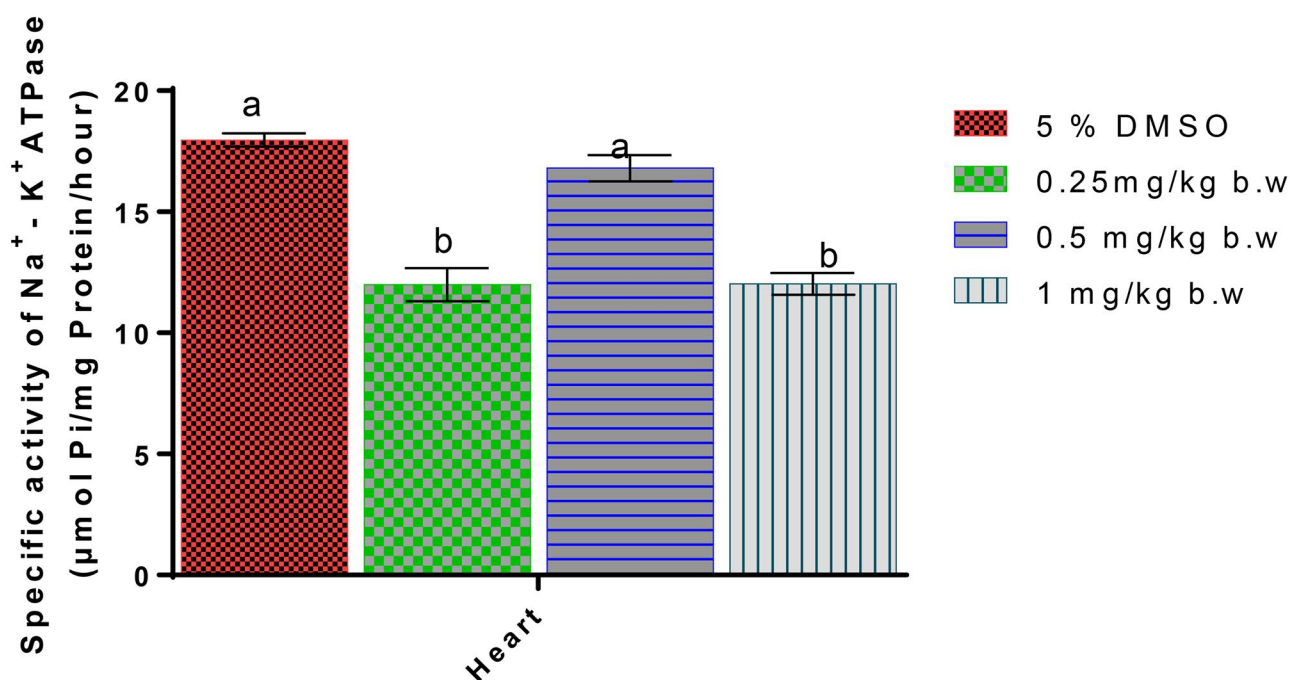


Fig. 9 Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on Na^{+} , K^{+} ATPase activity in heart of mice. Values are expressed as means of five replicates $\pm$ SEM.

Bars with different superscripts are significantly ($p < 0.05$) different from each other

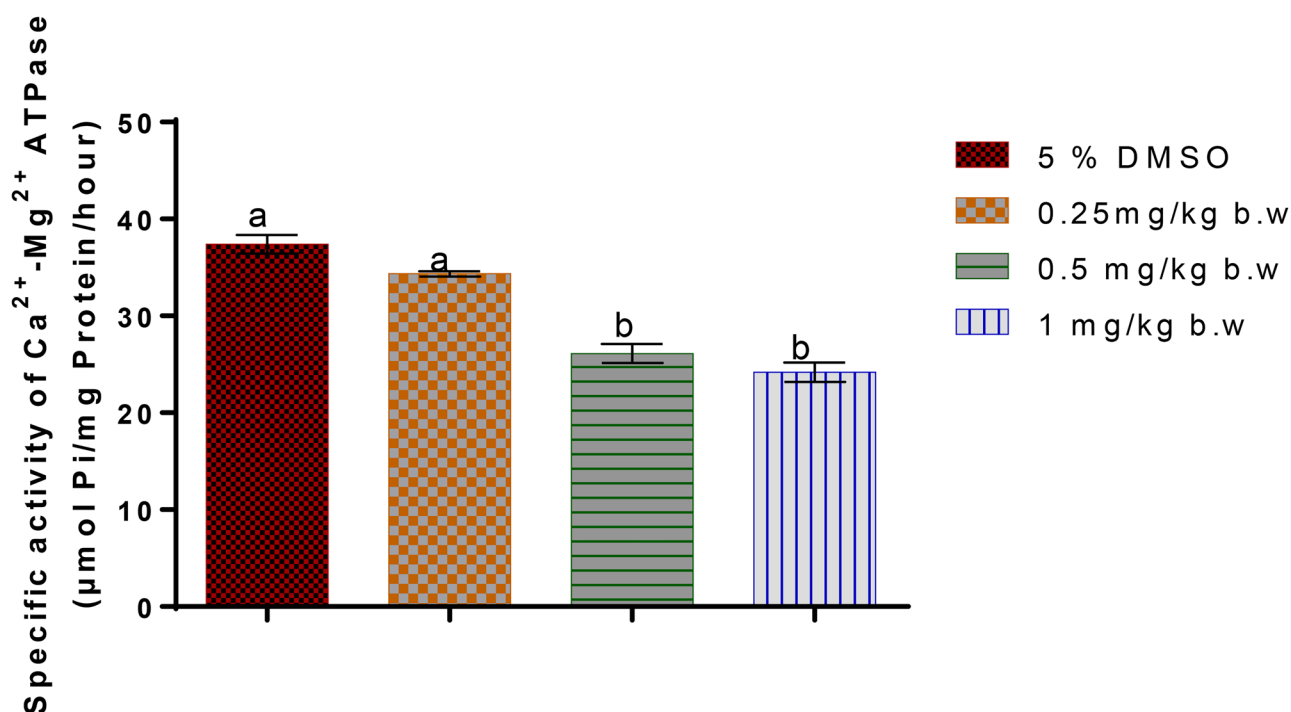


Fig. 10 Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on Ca^{2+} - Mg^{2+} activity in the heart of albino mice. Values are expressed as means of five repli-

cates $\pm$ SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

cytoplasm upon membrane excitation to induce contraction. The cardiac SR Ca^{2+} ATPase (SERCA2a) is encoded by the (human nomenclature) ATP2A2 gene, which also yields the SERCA2b isoform found in the endoplasmic

reticulum of most cells (Lytton et al. 1989; Zarain-Herzberg et al. 1990). Many experimental and clinical studies have indicated that inadequate function of the Ca^{2+} ATPase (SERCA2) and the consequent alterations of cytosolic Ca^{2+}

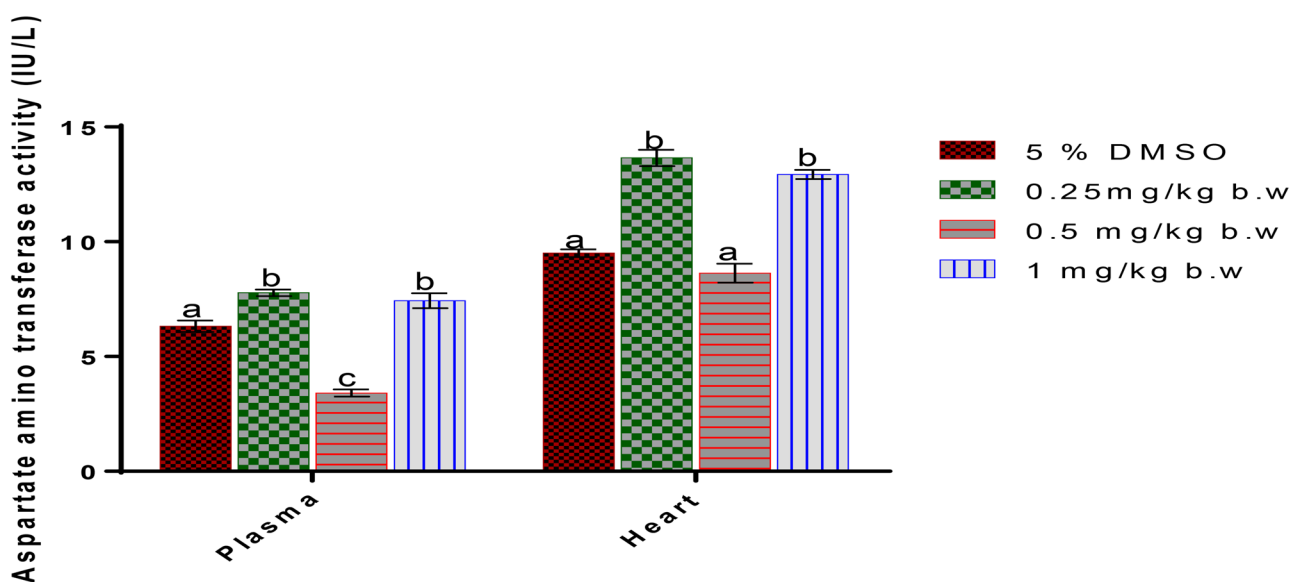


Fig. 11 Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on aspartate aminotransferase activity in the plasma and heart of mice. Values are expressed as means

of five replicates $\pm$ SEM. Bars for heart/plasma with different superscripts are significantly ($p < 0.05$) different from each other

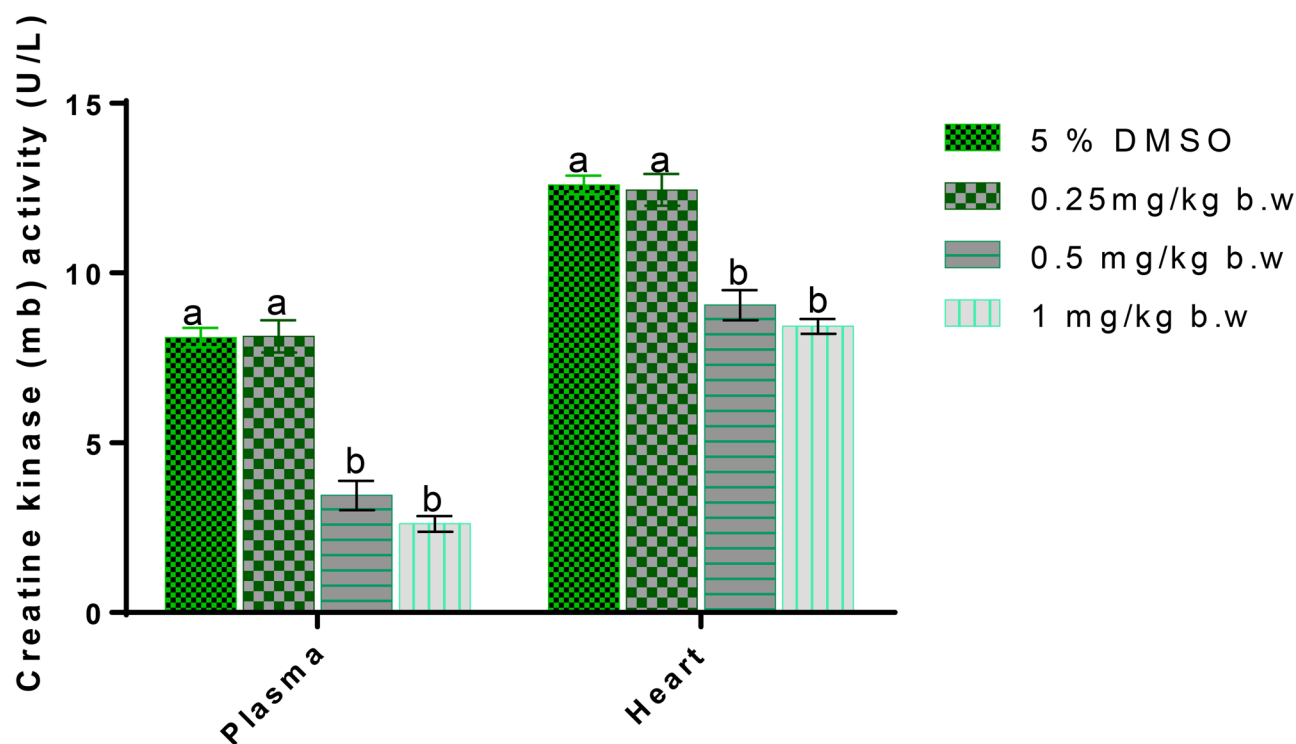


Fig. 12 Effects of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on Creatine kinase (MB) activity in the plasma and heart of albino mice. Values are expressed as mean

of five replicates $\pm$ SEM. Bars for the heart/plasma with different superscripts are significantly ($p < 0.05$) different from each other

signalling are pathological features of cardiac hypertrophy and failure (Bers et al. 2003; Chien 2000; Mercadier et al. 1990; Vangheluwe et al. 2006). The results revealed that there was reduction in Ca^{2+} ATPase activity in the groups administered 0.5 and 1 mg/kg body weight of 3DMACM. This may adversely affect the contraction/relaxation of cardiac muscle, most especially by hampering the sequestration of Ca^{2+} from the cytoplasm which may lead to various cardiovascular disorders, possibly through hardening of the cytoskeleton of the cells and impairment of the role of Ca^{2+} as a second messenger.

Mg^{2+} ATPase has been reported to function in the maintenance of cell membrane permeability and shape (Duncan 1967; Mircevova 1974). Membrane Mg^{2+} ATPase activity has been reported to be inversely related to lipid fluidity (Riordan 1980). Mg^{2+} ATPase activity of the actomyosin subsegment 1 has been reported to be a more reliable physiological indicator of cardiac muscle contractility (Lauer et al. 1989). In this study, heart Mg^{2+} ATPase activity was reduced by the compound compared to control, suggesting that the membrane fluidity was enhanced by the compound. Thus, increase in membrane fluidity above the optimum required may also account for the reduced activities of Ca^{2+} ATPase and $\text{Na}^{+}/\text{K}^{+}$ -ATPase, being membrane bound

enzymes, thereby leading to low contractility of the cardiac muscle.

The CK-MB (creatine kinase-muscle/brain) is a marker enzyme used for the diagnosis of acute myocardial infarction. Its activity in the blood represents the combination of two variants (isoenzymes CKM and CKB) of creatine kinase. There are 3 isoenzymes of creatine kinase (CK)-BB, -MM, and -MB. The primary source of CKMB is the myocardium, although it is also found in skeletal muscle. CKMB levels increase with myocardial damage. There was reduction in CK-MB activity in both the plasma and heart of the groups administered 0.5 and 1 mg/kg body weight of compound compared to controls. This suggests inhibition of the enzyme in situ or suppression of its synthesis. This may adversely affect the formation of creatine phosphate which serves as energy reserve for muscle contraction. Depletion of creatine phosphate may adversely affect the regeneration of ATP which is required during muscle contraction and relaxation.

Lactate dehydrogenase (LDH) is an enzyme required during anaerobic glycolysis. It is used as a marker enzyme for myocardial infarction. LDH activity increases in blood when there is myocardial infarction. In this study, the compound reduced plasma LDH at all doses administered, suggesting inhibition of the enzyme in situ.

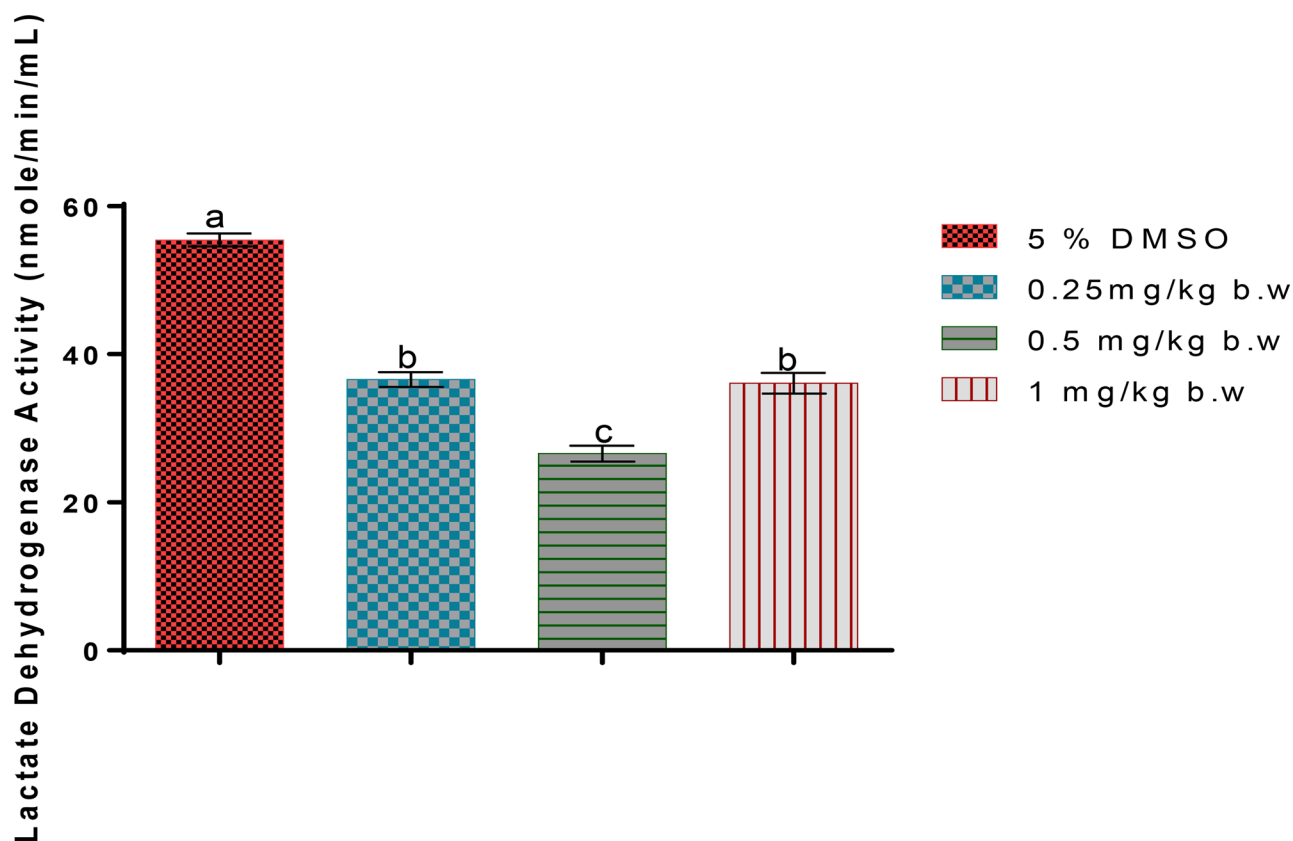


Fig. 13 Effect of 3-O-[6-deoxy-3-O-methyl- β -D-allopyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl]-17 β -marsdenin on Lactate Dehydrogenase activity in the plasma of mice. Values are expressed as means of five repli-

cates $\pm$ SEM. Bars with different superscripts are significantly ($p < 0.05$) different from each other

Haematological parameters

Inflammation plays a crucial pathophysiological role in the entire continuum of the atherosclerotic process, from its initiation, progression, and plaque destabilization leading

ultimately to an acute coronary event. Furthermore, once the clinical event has occurred, inflammation also influences the left ventricular remodelling process. Under the same paradigm, there is evidence that lymphocytes play an important role in the modulation of the inflammatory

Table 1 Haematological parameters of mice administered 3-O-[6-deoxy-3-O-methyl- β -D- allopyranosyl-(1 4)- β -D-canaropyranosyl]-17 β -marsdenin

Treatment	Hb (g/dl)	HCT (%)	RBC ($\times 10^{12}/L$)	MCV (fL)	MCH (pg)	MCHC (g/dL)	WBC ($\times 10^9/L$)	LYM (%)	PLT ($\times 10^9/L$)
5% DMSO	11.8 $\pm$ 0.33a	53.07 $\pm$ 0.85a	9.08 $\pm$ 0.16a	58.37 $\pm$ 0.65a	13.04 $\pm$ 0.30a	21.68 $\pm$ 0.37a	15.33 $\pm$ 0.70a	94.78 $\pm$ 0.49a	361.00 $\pm$ 34.00a
0.25 mg/kg body weight	12.17 $\pm$ 0.20b	54.47 $\pm$ 1.57a	9.55 $\pm$ 0.32a	58.30 $\pm$ 0.75a	12.50 $\pm$ 0.11a	20.94 $\pm$ 0.30a	12.57 $\pm$ 0.79a	90.97 $\pm$ 1.62b	348.33 $\pm$ 20.00b
0.5 mg/kg body weight	11.96 $\pm$ 0.46a	55.93 $\pm$ 1.48a	8.43 $\pm$ 0.48b	57.87 $\pm$ 0.96a	12.78 $\pm$ 0.37a	21.68 $\pm$ 0.51a	11.40 $\pm$ 0.80a	90.98 $\pm$ 1.85b	312.67 $\pm$ 21.37c
1 mg/kg body weight	11.83 $\pm$ 0.45a	47.65 $\pm$ 1.95b	8.62 $\pm$ 0.58b	61.50 $\pm$ 0.66b	13.03 $\pm$ 0.24a	21.58 $\pm$ 0.42a	13.60 $\pm$ 0.46a	89.63 $\pm$ 3.19b	298.50 $\pm$ 83.50d

Values are expressed as means of five replicates $\pm$ SEM. Values in each column with different letters are significantly ($p < 0.05$) different from each other

WBC white blood cell count, LYM percentage lymphocyte, PLT platelet, RBC red blood cell count, Hb haemoglobin, PCV packed cell volume, MCV mean corpuscular volume, MCH mean corpuscular haemoglobin, MCHC mean corpuscular haemoglobin concentration

response at every level of the atherosclerotic process. Low lymphocyte count (LLC) is a common finding in systemic inflammatory response during atherosclerotic process in both clinical and animal studies, which suggests that LLC plays a putative role in accelerating atherosclerosis (Libby 2012). For instance, there is evidence that LLC is associated with worse outcomes in patients with heart failure, chronic ischemic heart disease, and acute coronary syndromes (Nuñez et al. 2011). Thus, the reduced percentage lymphocyte observed at all doses of the compound in this study compared to control (Table 1) suggests that the compound has the potential of enhancing the suppression of the adaptive immune system, which has been associated with the acceleration of the development of atherosclerosis. However, since the observed percentage lymphocyte is within the normal range, it may not enhance the development of atherosclerosis.

Platelets may be abnormal either quantitatively (too many or too few) or qualitatively (the right number but they do not work correctly). Normal platelet count ranges from 150,000 to 450,000 (Msaouel et al. 2014). Platelets play a vital role in atherogenesis (Patzelt et al. 2015). They interact with the endothelial membrane before the formation of atherosclerotic plaque, thereby forming the focal point for immune cell-driven inflammation associated with atherosclerosis (Gawaz et al. 2005; Massberg et al. 2002). In this study, the compound reduced platelet count at all doses, though not below the normal range, compared to control (Table 1). The result suggests that the compound has the potential of impairing platelet formation, thereby impairing their role in atherogenesis.

The results obtained in this study revealed that the compound reduced red blood cell count at higher doses compared to control (Table 1). This suggests that the compound may cause haemolysis and reduce the oxygen carrying capacity of the blood. This is further evidenced by the haematocrit which was significantly reduced at the highest concentration administered compared to control. However, there was increased MCV at the highest dose of the compound, suggesting increased size of red blood cells formed, though without reduced concentration of haemoglobin compared to control.

Conclusion

The results suggest that 3DMACM may not predispose subjects to atherosclerosis but may adversely affect cardiac muscle contraction/relaxation and other signalling pathways which are triggered by calcium which serves as a second messenger. All these, coupled with other alterations, may be the onset of other types of cardiovascular diseases.

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Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Ethics approval The study was carried out in accordance with the 1996 guide for the care and use of laboratory animals ILAR Journal 38:41–48.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

- Abolaji A, Adebayo H, Odesanmi O (2007) Effects of ethanolic fruit extract of *Parinari polyandra* (Rosaceae) on serum lipid profile and some electrolytes in pregnant rabbits. *Res J Med Plant* 1:121–127
- Ahmad M, Medford RM (1995) Evidence for the regulation of Na⁺, K⁺-ATPase α 1 gene expression through the interaction of aldosterone and cAMP-inducible transcriptional factors. *Steroids* 60(1):147–152
- Akanji MA, Olagoke OA, Oloyede OB (1993) Effect of chronic consumption of metabisulphite on the integrity of the rat kidney cellular system. *Toxicology* 81:173–179
- Akinnuga AM, Bamidele O, Ekechi P, Adeniyi OS (2011) Effects of an ethanolic leaf extract of *Gongronema latifolium* on some Haematological parameters in rats. *Afr J Biomed Res* 14:153–156
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. *Clin Chem* 20:470–475
- Bachorik PS, Walker R, Brownell KD, Stunkard AJ, Kwiterovich PO (1980) Determination of high density lipoprotein-cholesterol in stored human plasma. *J Lipid Res* 21:608–616
- Bagrov AY, Shapiro JI (2008) Endogenous digitalis: pathophysiologic roles and therapeutic applications. *Nat Clin Pract Nephrol* 4:378–392
- Bagrov AY, Shapiro JI, Fedorova OV (2009) Endogenous cardiotonic steroids: physiology pharmacology and novel therapeutic targets. *Pharmacol Rev* 61:9–38
- Berry RG, Despa S, Fuller W, Bers DM, Shattock MJ (2007) Differential distribution and regulation of mouse cardiac Na⁺/K⁺-ATPase α 1 and α 2 subunits in T-tubule and surface sarcolemmal membranes. *Cardiovasc Res* 73:92–100
- Bers DM, Eisner DA, Valdivia HH (2003) Sarcoplasmic reticulum Ca²⁺ and heart failure: roles of diastolic leak and Ca²⁺ transport. *Circ Res* 93(6):487–490
- Beshel JA, Beshel FN, Nku CO, Owu DU (2019) *Gongronema latifolium*: A plant with cardioprotective potentials. *Int J Trend Sci Res Dev* 3(2):548–558
- Bewaji CO, Olorunsogo OO, Bababunmi EA (1985) Comparison of the membrane-bound (Ca²⁺ + Mg²⁺)-ATPase in erythrocyte ghosts from some mammalian species. *Comp Biochem Physiol B Comp Biochem* 82:117–122

- Burnier M (2007) Sodium in health and disease. CRC Press, Boca Raton
- Chien KR (2000) Meeting Koch's postulates for calcium signaling in cardiac hypertrophy. *J Clin Invest* 105:1339–1342
- Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF (1997) The 1996 guide for the care and use of laboratory animals. *ILAR J* 38:41–48
- Cui X, Xie Z (2017) Protein interaction and Na/K-ATPase-mediated signal transduction. *Molecules* 22:990
- Deans KA, Dominiczak MH (2008) Hyperlipidaemia and cardiovascular disease: apolipoprotein measurements and metabolic syndrome as predictors of cardiovascular risk. *Curr Opin Lipidol* 19:624–626
- Desfrere L et al (2009) Na K-ATPase signal transduction triggers CREB activation and dendritic growth. *Proc Natl Acad Sci* 106:2212–2217
- Duncan CJ (1967) The molecular properties and evolution of excitable tissues. Pergamon Press, Oxford
- Dzoyem JP, Kuete V, Eloff JN (2014) Biochemical parameters in toxicological studies in Africa: significance principle of methods data interpretation and use in plant screenings. In: Kuete V (ed) *Toxicological Survey of African Medicinal Plants*. Elsevier, Amsterdam, pp 659–715
- Edet E, Akpanabiatu M, Uboh F, Edet T, Eno A, Itam E, Umoh I (2011a) Gongronema latifolium crude leaf extract reverses alterations in hematological indices and weight loss in diabetic rats. *J Pharmacol Toxicol* 6(2):174–181
- Edet E, Atangwho I, Akpanabiatu M, Edet T, Uboh F, David-Oku E (2011b) Effect of Gongronema latifolium leaf extract on some liver enzymes and protein levels in diabetic and non-diabetic rats. *J Pharm Biomed Sci* 1(5):104–107
- Edet E, Eno MAA, Umoh I, Itam E (2009) Effect of Gongronema latifolium crude leaf extract on some cardiac enzymes of alloxan-induced diabetic rats. *Afr J Biochem Res* 3(11):366–369
- Erecinska M, Silver IA (1989) ATP and Brain Function. *J Cerebral Blood Flow Metab* 9:2–19
- Fleschner CR, Kraus-Friedmann N (1986) The effect of Mg²⁺ on hepatic microsomal Ca²⁺ and Sr²⁺ transport. *Eur J Biochem* 154:313–320
- Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502
- Garber JC (2011) On the care and use of US lab animals. *Nature* 476:152
- Gawaz M, Langer H, May AE (2005) Platelets in inflammation and atherogenesis. *J Clin Invest* 115:3378–3384
- Gornall AG, Bardawill CJ, David MM (1949) Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 177:751–766
- Gyebi GA et al (2018) Iloneoside: a cytotoxic ditigloylated pregnane glycoside from the leaves of *Gongronema latifolium* Benth. *Nat Prod Res* 32:2882–2886
- Jensen LJ, Salomonsson M, Jensen BL, Holstein-Rathlou NH (2004) Depolarization-induced calcium influx in rat mesenteric small arterioles is mediated exclusively via mibefradil-sensitive calcium channels. *Br J Pharmacol* 142:709–718
- Lauer B, Van Thiem N, Swynghedauw B (1989) ATPase activity of the cross-linked complex between cardiac myosin subfragment 1 and actin in several models of chronic overloading: A new approach to the biochemistry of contractility. *Circ Res* 64:1106–1115
- Libby P (2012) Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* 32:2045–2051
- Liu J, Xie Z-J (2010) The sodium pump and cardiotonic steroids-induced signal transduction protein kinases and calcium-signaling microdomain in regulation of transporter trafficking. *Biochim Biophys Acta Mol Basis Dis* 1802:1237–1245
- Lytton J, Zarain-Herzberg A, Periasamy M, MacLennan D (1989) Molecular cloning of the mammalian smooth muscle sarco (endo) plasmic reticulum Ca²⁺-ATPase. *J Biol Chem* 264:7059–7065
- Malomo S (2000) Toxicological implication of ceftriaxone administration in rats. *Nig J Biochem Mol Biol* 15:33–38
- Massberg S et al (2002) A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med* 196:887–896
- Mercadier J-J et al (1990) Altered sarcoplasmic reticulum Ca²⁺ (+)-ATPase gene expression in the human ventricle during end-stage heart failure. *J Clin Invest* 85:305–309
- Miao Y, Xu X, Yuan F, Shi Y, Chen Y, Chen J, Li X (2016) Four cytotoxic annonaceous acetogenins from the seeds of *Annona squamosa*. *Nat Prod Res* 30:1273–1279
- Mircevaova L (1974) Scanning electron microscopy of erythrocyte ghost prepared with and without ATP addition. *B Lit* 29:108–144
- Mohammed A, Ibrahim MA, Islam MS (2014) African medicinal plants with antidiabetic potentials: a review. *Planta Med* 80:354–377
- Moore KL, Dalley AF (2018) Clinically oriented anatomy. Wolters Kluwer India Pvt Ltd, Gurgaon
- Morebise O (1998) Antimicrobial and phytotoxic activities of saponin extract from two Nigerian edible medicinal plants. *Biokemistri* 8:69–77
- Morebise O, Fafunso M, Makinde J, Olajide O (2006) Evaluation of the bioactivity of Gongronema latifolium leaf extract in rodents. *Sci Focus* 11:27–30
- Msaouel P et al (2014) Abnormal platelet count is an independent predictor of mortality in the elderly and is influenced by ethnicity. *Haematologica* 99:930–936
- Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 75:311–335
- Nicholls SJ, Tuzcu EM, Nissen SE (2007) Atherosclerosis regression: is low-density lipoprotein or high-density lipoprotein the answer? *Curr Atheroscler Rep* 9:266–273
- Núñez E, Martínez P, Cuenca V, García FJ, Moreno D, Urda A (2011) Coronary artery abnormalities in Kawasaki disease according to patient age. *Pediatr Rheumatol* 9:P86
- Olukanni O, Akande O, Alagbe Y, Adeyemi O, Olukann A, Daramola G (2013) Lemon juice elevated level of reduced glutathione and improved lipid profile in Wistar rats American-Eurasian. *J Agric Environ Sci* 13:1246–1251
- Okpala J, Sani I, Abdullahi R, Ifedilichukwu H, Igwe J (2014) Effects of n-butanol fraction of Gongronema latifolium leave extract on some biochemical parameters in CCl₄-induced oxidative damage in Wistar albino rats. *Afr J Biochem Res* 8(2):52–64
- Patzelt J, Verschoor A, Langer HF (2015) Platelets and the complement cascade in atherosclerosis. *Front Physiol* 6:49
- Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 28:56–63
- Riordan JR (1980) Plasma membrane Mg²⁺ ATPase activity is inversely related to lipid fluidity. In: Kates M, Kuksis A (eds) *Membrane fluidity*. Springer, Switzerland, pp 119–129
- Ronner P, Gazzotti P, Carafoli E (1977) A lipid requirement for the (Ca²⁺++ Mg²⁺)-activated ATPase of erythrocyte membranes. *Arch Biochem Biophys* 179:578–583
- Schneider C, Rotscheldt K, Breitmaier E (1993) Vier neue pregnanglycoside aus Gongronema latifolium (Asclepiadaceae). *Liebigs Ann Chem* 1993:1057–1062
- Stamler J, Wentworth D, Neaton JD (1986) Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded?: findings in 356 222 primary

- screenees of the multiple risk factor intervention trial (mrfit). *JAMA* 256:2823–2828
- Tietz NW (1990) Clinical guide to laboratory tests. 2nd Edn. WB Saunders Company, Philadelphia, USA, pp 554–556
- Vander AJ, Sherman JH, Luciano DS (1998) Human physiology: the mechanisms of body function, vol 612 V228h. McGraw-Hill, New York
- Vangheluwe P, Sipido K, Raeymaekers L, Wuytack F (2006) New perspectives on the role of SERCA2's Ca²⁺ affinity in cardiac function. *Biochim Biophys Acta Mol Cell Res* 1763:1216–1228
- Weisshaar H, Prasad M, Parker R (1975) Estimation of lactate dehydrogenase in serum/plasma. *Med Welt* 26:387
- Witt I, Trendelenburg C (1982) Joint study to establish reference values for clinical chemical parameters in childhood (author's transl). *J Clin Chem Clin Biochem Z Klin Chem Klin Biochem* 20:235–242
- Wright PJ, Ngaha EO, Plummer DT (1974) The effect of cephaloridine on enzyme excretion into the urine. Portland Press Ltd., London
- Yakubu M, Akanji M, Oladiji A (2007) Hematological Evaluation in Male Albino Rats following Chronic Administration of Aqueous Extract of *Fadogia Agrestis* Stem. *Pharmacogn Mag* 3:34
- Zarain-Herzberg A, MacLennan D, Periasamy M (1990) Characterization of rabbit cardiac sarco (endo) plasmic reticulum Ca²⁺ (+)-ATPase gene. *J Biol Chem* 265:4670–4677

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