Cardio-protective Effects of Aqueous Extracts of Garlic and Ginger in Laboratory Animals

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Abstract- Garlic and ginger have attracted particular attention of modern medicine because of its wide spread use around the world and the cherished belief that it helps to maintain good health by warding off illness and providing more vigor. The study investigated the cardio-protective property of Garlic *(Allium sativum)* and Ginger *(Zingiber officiale)* extracts in laboratory animals. Animals of both sexes were assigned into the following: Group 1: Served as control and were administered with 1ml of distilled water, Group 2: Received 20mg/kg b w of garlic, Group 3: Received 40mg/kg b w of garlic, Group 4: Received 20mg/kg b w of ginger, Group 5: Received 40mg/kg b w of ginger, Group 6: Received garlic 10mg and ginger 10mg/kg b w Group 7: Received garlic 20mg and ginger 20mg/kg b w. All administration was given orally once daily for a duration of four (4) weeks. Animals were euthanized after the last day of extract treatment and blood samples were collected and serum separated for lipid profile assay. The results obtained in the study showed a statistically significant reduction ($p<0.05$) of serum total cholesterol, triglyceride and low density lipoprotein levels at all single and combined doses of the extract administered to the animals when compared to the control group. However, the extract at single and combined doses significantly elevated ($p<0.05$) serum high density lipoprotein level in the treated animals when compared the control group. The administration of the extract especially at the combined doses produced a reduction on the rate and force of contraction respectively when compared with the baseline line control level The interaction between the extract and the standard drug (adrenaline) revealed that the extract completely blocked the action of adrenaline when co-administered, hence reducing both the rate and force of contraction . In conclusion, the findings suggest that the extract as well as its combination improves lipid profile in the animals and the cardio-protective effect of garlic and ginger was linked to the decrease on the rate and force of contraction of isolated perfuse heart studied.

Key words: Garlic; ginger; isolated perfuse heart; total cholesterol; triglyceride; low-density lipoprotein

1. Introduction

Garlic *(Allium Sativum)* and ginger *(Zingiber officiale)* are widely consumed spices in food and drink form. Several studies have been done on these plants separately especially on the heart and blood pressure. But there is no record of their combined effects. However these plants are often consumed in combined form as food spices. Human and animal studies have substantiated that garlic and ginger lowers serum cholesterol and triglycerides and increases the amount of high-density lipoproteins (HDL) [1]. Garlic is a popular supplement well-perceived as a healthy choice among people looking to increase cardiovascular wellness. Approximately 4% of all cardiovascular disease patients and 30% of cardiovascular patients who use herbal supplements take garlic [2]. Known risk factors for cardiovascular disease include inflammation, high cholesterol, high homocysteine, high blood pressure, diabetes and dementia, including its most common form, Alzheimer's disease [3]. Garlic also reduces cholesterol synthesis by

inhibiting 3-hydroxy-3-methylglutaryl-CoA. Garlic has been shown to inhibit LDL oxidation, platelet aggregation, arterial plaque formation, decrease homocysteine, lower blood pressure, and increase micro circulation [4]. Recent in vitro studies by Benavides *et al.,* [5] have confirmed the vasoactive ability of garlic's sulfur compounds whereby red blood cells convert garlic's organic polysulfides into hydrogen sulfide, a known endogenous cardio-protective vascular cell signaling molecule. In traditional Chinese medicine, ginger is used to improve the flow of body fluids. It stimulates blood circulation throughout the body by powerful stimulatory effect on the heart muscle and by diluting blood [6]. Japanese study showed that active constituents in ginger reduced the blood pressure and decreased cardiac work load [7]. Several reports, mainly from rat studies, have suggested that ginger exerts many direct and indirect effects on blood pressure and heart rate [8]. In Guinea pig paired atria, the crude extract exhibited a cardio-depressant activity on the rate and force of spontaneous contractions [9]. The present study aimed at investigating the cardio-protective activity of

Ginger *(Zingiber officiale)* and Garlic *(Allium sativum)* in laboratory animals.

2. Materials and Methods

2.1 Collection and preparation of plant extract

The fresh plant of garlic and ginger were purchased from Samaru market in Zaria, Kaduna State and dried under shade and then ground into fine powder using laboratory mortar and pestle. The powder (150g) of garlic and (208g) of ginger was macerated in cold water at room temperature for 24 hours. This was then filtered using a filter paper and the filtrate was evaporated to dryness on water bath at 40 ^{0}C to a dry residue of (24g) of garlic and (34g) of ginger and kept in an air tight bottle until they were reconstituted for administration.

2.2 Animals

Strains of albino wistar rats of both sexes between the ages of 12 - 16 weeks old and weighing between 150-200 g were procured from Department of pharmacology and therapeutics animal house. The animals were kept in well aerated laboratory cages in the Department of Human physiology animal house and were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. They were maintained on standard animal feeds and drinking water *ad libitum* during the acclimatization period.

2.3 Experimental design

In the study thirty five (35) wistar rats were used. Each group consisted of five animals, and they were divided into the following groups as follows:

Group 1: Served as control and were administered with 1ml of distilled water orally.

Group 2: Received 20mg/kg body weight of garlic orally

Group 3: Received 40mg/kg body weight of garlic orally

Group 4: Received 20mg/kg body weight of ginger orally.

Group 5: Received 40mg/kg body weight of ginger orally.

Group 6: Received garlic 10mg and ginger 10mg/kg body weight orally

Group 7: Received garlic 20mg and ginger 20mg/kg body weight orally.

The regimens were given to the animals once daily spanning for a period of four (4) weeks.

2.4 Collection and preparation of sera samples for lipid profile assays

Following the last day of treatment all animals were euthanized by exposing them to over dose of chloroform soaked in cotton wool placed in anesthetic box covered with lid. Blood samples were drawn from the heart of each sacrificed animal from all groups by cardiac puncture after they have been fasted for 12 hours. Blood samples was collected were collected in plain tubes and were allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 1968 g for 15 minutes and subjected for lipid profile assay.

2.5 Determination of lipid profile

2.5.1 Determination of serum total cholesterol

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein [10]. 1000 μl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A $_{sample}$) and standard (A $_{standard}$) was measured against the reagent blank within 30 minutes at 546nm. The value of TC present in serum was expressed in the unit of mg/dl.

2.5.2 Determination of serum triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz [11]. 1000 μl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A $_{sample}$) and standard (A $_{standard}$) was measured against the reagent blank within 30 minutes at 546nm.The value of triglyceride present in the serum was expressed in the unit of mg/dl.

2.5.3 Determination of serum high-density lipoprotein cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Albers [12]. Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in the unit of mg/dl.

2.5.4 Determination of serum low-density lipoprotein cholesterol

The serum level of (LDL-C) was measured according to protocol of Friedewald *et al*., [13] using the relationship below:

LDL-C = TC-TGL/5 + HDL-C

The value was expressed in the unit of mg/dl.

2.6 Evaluation of Effects of garlic and ginger on isolated rabbit heart

This was carried out by method described by Zimmer [14]. Briefly, the rabbit was sacrificed by a blow on the head. The heart was promptly excised and transferred to the petri dish tyrode solution. The aorta was quickly dissected free of all its branches and the heart was mounted on the apparatus where it was tied to a glass cannula through which it receives its nourishment. The heart tissue was properly nourished with oxygen using an oxygenated which was constantly perfusing the physiological solution with oxygen. This helps keep the heart alive. The temperature of the whole system was maintained at 37°C. A thread was attached to the ventricle by the hook which was connected to a series of lever and then to the transducer which was then connected to the writing pen which recorded the contraction on kymograph model Number 7050 at speed of 24mm/min. The rate of beating was measured from the tracing with a tracing of sixty (60) seconds each. The baseline or steady control level was determined before the commencement of the experiment. The extract was administered and at each treatment the heart was allowed to recover o reach a basal contraction before the next administration.

2.7 Statistical analysis

The results obtained were represented as mean ± SEM and statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software. The values of p<0.05 was considered significant.

3. Results

Effects of Garlic and Ginger on serum lipid profile

There was a statistically significant reduction (p<0.05) of serum total cholesterol, triglyceride and low density lipoprotein levels at all single and combined doses of the extract administered to the animals when compared to the control group. However, the extract at single and combined doses significantly elevated (p<0.05) serum high density lipoprotein level in the treated animals when compared the control group (Table 1).

Effects of Garlic and Ginger on isolated rabbit heart

Figure 1 shows the baseline or steady control level of contraction of isolated rabbit heart when treated with normal saline. However, administration of the extract especially at the combined doses produced a reduction on the rate and force of contraction respectively when compared with the baseline line control level (figure 4). The interaction between the extract and the standard drug (adrenaline) revealed that the extract completely blocked the action of adrenaline when co-administered; hence reducing both the rate and force of contraction (figure 5)

Table 1: Effects of Garlic (Allium Sativum) and Ginger (Zingiber officinale) on mean (±SEM) serum lipid profile in wistar rats

Treatment Given	Serum total cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Serum low density lipoprotein (mg/dl)	Serum high density lipoprotein (mg/dl)
Normal + Distilled Water	106.97 ± 2.41	86.14 ± 4.15	64.16 ± 4.23	21.57 ± 1.91
Garlic 20mg/kg b w	62.49 ± 4.09 [a]	48.09 ± 5.96 [a]	22.63 ± 2.24 [a]	31.64 ± 3.40 [a]
Garlic 40mg/kg b w	72.64 ± 4.65 [a]	49.54 ± 4.31 [a]	19.84 ± 1.77 [a]	42.89 ± 4.27 [a]
Ginger 20mg/kg b w	59.24 ± 4.09 [a]	27.66 ± 2.63 [a]	11.81 ± 1.13 [a]	41.97 ± 3.56 [a]
Ginger 40mg/kg b w	58.17 ± 5.83 [a]	31.24 ± 5.67 [a]	15.06 ± 1.44 [a]	37.40 ± 3.51 [a]
Garlic 10mg + Ginger 10mg/kg b w	51.27 ± 5.29 [a]	30.00 ± 6.45 [a]	13.49 ± 9.28 [a]	31.79 ± 2.54 [a]
Garlic 20mg + Ginger 20mg/kg b w	81.86 ± 12.35[a]	27.20 ± 7.16 [a]	13.69 ± 2.28 [a]	62.73 ±12.23 [a]

[a] p < 0.05 is statistically significant when compared to control group while ns=not significant
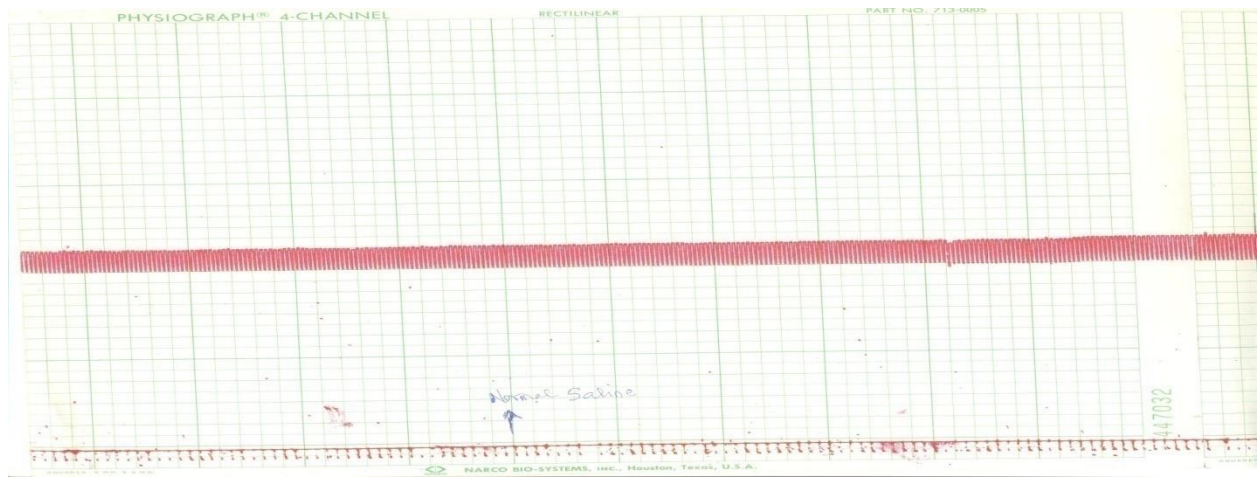
Figure 1: Baseline or steady control level of contraction of isolated rabbit heart when administered with normal saline
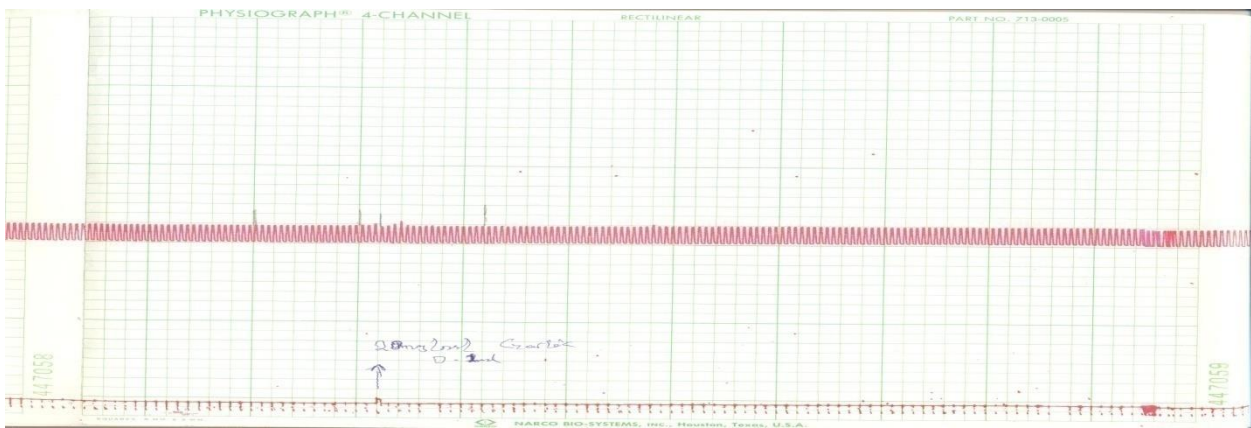

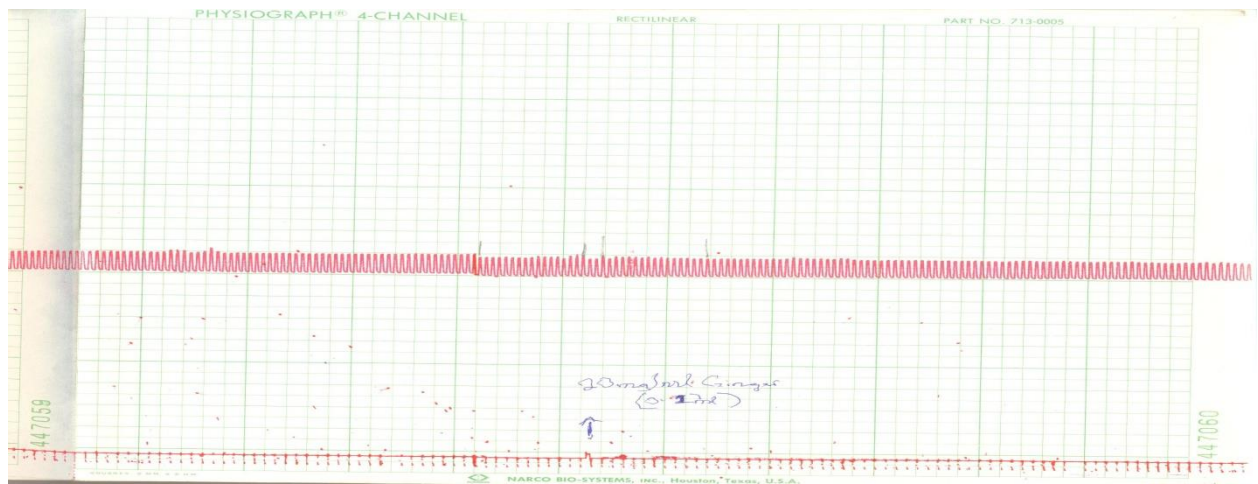**Figure 2:** Effect of aqueous extract of garlic (20mg·ml-1) on isolated rabbit heart

Figure 3: Effect of aqueous extract of ginger (20mg·ml-1) on isolated rabbit heart
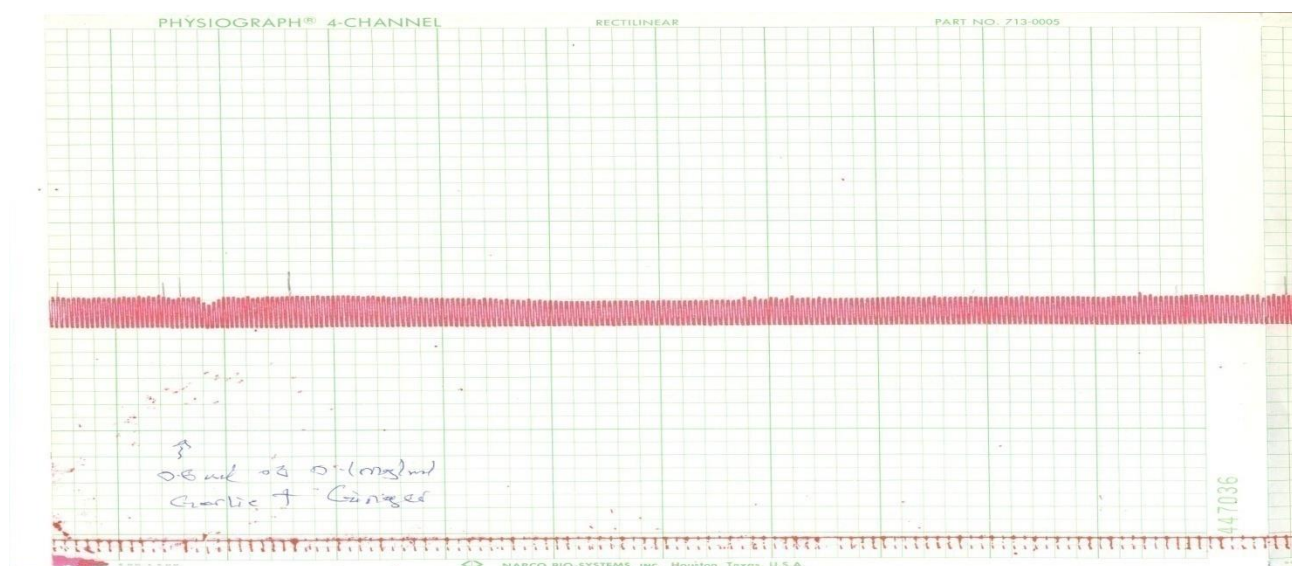
Figure 4: Effect of aqueous extract of garlic (0.1mg·ml-1) and ginger (0.1mg·ml-1) on isolated rabbit heart
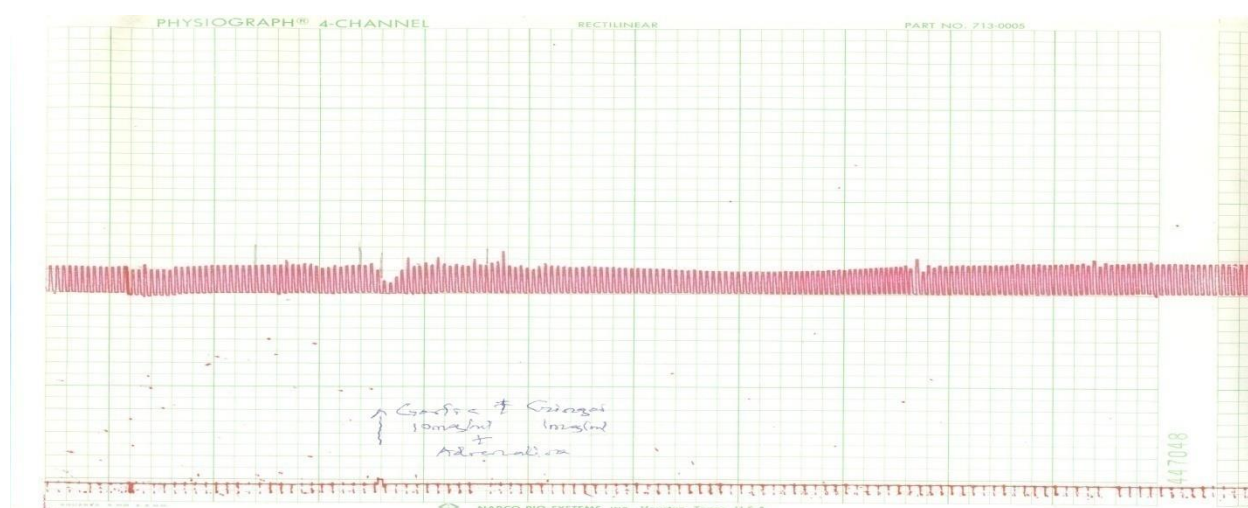

Figure 5: Interaction of aqueous extract of garlic (1mg·ml-1) and ginger (1mg·ml-1) and adrenaline (1 µg/ml) on isolated rabbit heart

4. Discussion

Diseases mainly affecting the heart or blood vessels are primarily termed as cardiovascular diseases (CVDs). Today, CVDs constitute one of the major causes of mortality and have become a major health hazard all over the world since they account for more than 30% of the global deaths every year [15] - [16]. Hyperlipidaemia is an important risk factor of coronary artery diseases which often lead to myocardial infarction and heart failure [17] – [18]. The present study investigated the cardio-protective activity of garlic and ginger extract and it combination in laboratory animals. Management of plasma cholesterol levels continues to be a cardinal issue in cardiovascular disease prevention, as hypercholesterolemia plays a crucial role in pathogenesis of atherosclerosis and related heart diseases [19] – [20]. The results showed that there was a statistically significant reduction of serum total cholesterol concentration in the animals administered both single and combined doses of aqueous garlic and ginger extract when compared to the control group. Allicin has been proposed as the active compound produced by garlic which is responsible for its hypocholesterolemic effect [21]. Garlic and ginger have been reported to modify lipid metabolism by inhibiting cellular cholesterol biosynthesis, increasing bile acid biosynthesis to eliminate cholesterol from the body and increasing fecal cholesterol excretion, therefore, cholesterol synthesis is reduced by up to 75% [22] – [27] The cholesterol lowering effects of garlic has also been attributed not only to its organosulfur constituents but also to a variety of steroidal saponins present in garlic extract [28]. There was also significantly decreased serum triglyceride and low density lipoprotein levels at all single and combined doses of the aqueous garlic and ginger administered to animals when compared to the control group. However, the extract at single and combined doses significantly elevated (p<0.05) serum high density lipoprotein level in the treated animals when compared the control group. These findings are in agreement with Ali *et al.,* [28] who suggested that administration of garlic to rats is effective

in decreasing total cholesterol and triglycerides significantly. The mechanism for triglycerides lowering effect of garlic is not well understood. However, Yeh and Yeh, [29] demonstrated that the rate of acetate incorporation into fatty acid was reduced in hepatic cell culture treated with garlic extract. Thus, the triglycerides lowering effect of garlic may somehow be due to the inhibition of fatty acids synthesis. Elshater *et al.,* [30] revealed that post treatment with ginger extract for 6 weeks to diabetic rats produced significant reduction in the levels of plasma cholesterol, triglycerides and LDL-cholesterol and significant elevation in the HDL-cholesterol when compared with diabetic group. In another study, Alizadeh *et al.,* [31] investigated the effect of 45 days ginger capsules on the lipid levels in patients with hyperlipidemia and indicated that ginger has a significant lipid lowering effect when compared to placebo. The reduction in LDL-c level by garlic may be due to allicin an active compound produced by garlic which reduces the production and release of LDL-c by the liver and promote LDL receptors activity in the liver cells, which helps the liver to clear the circulating LDL-C [32]. Brousseau *et al.,*[33] reported that, the increase in HDL-C level is usually attributed to allicin, which significantly altered the distribution of cholesterol among HDL and LDL subclasses by a mechanism, appears to be due to a reduction of VLDL level, with a secondary decrease in apo D activity which results in less transfer of HDL-c to VLDL acceptor particles. Hyperlipidemia is the underlying pathophysiology of the number one killer, atherosclerotic coronary artery heart disease [1]. However, the present study revealed that administration of the extract especially at the combined doses produced a reduction on the rate and force of contraction respectively when compared with the baseline line control level. The interaction between the extract and the standard drug (adrenaline) revealed that the extract completely blocked the action of adrenaline when co-administered, hence reducing both the rate and force of contraction. This suggests that the extracts may be acting via β_1-adrenergic receptor located on the myocardium. The blockade of β_1 receptors has been shown to cause negative inotropic and chronotropic effect which in turn bring about decrease in cardiac work and cardiac output [34]. These effects are useful in the treatment of cardiac infarction, cardiac arrhythmias and angina pectoris. Recent in vitro studies by Benavides *et al.,* [5] have confirmed the vasoactive ability of garlic's sulfur compounds whereby red blood cells convert garlic's organic polysulphides into hydrogen sulfide, a known endogenous cardio-protective vascular cell signaling molecule. It stimulates blood circulation throughout the body by powerful stimulatory effect on the heart muscle and by diluting blood [6]. More recently, Ghayur and Gilani [35] reported that the crude extract of ginger induced a dose-dependent decrease in the arterial blood pressure of anesthetized rats. And in guinea pig paired atria, the crude extract exhibited a cardio-depressant activity on the rate and force of spontaneous contractions.

5. Conclusion

In conclusion, the findings suggest that the extract as well as its combination improves lipid profile in the animals and the cardio-protective effect of garlic and ginger was linked to the decrease on the rate and force of contraction of isolated perfuse heart studied.

Acknowledgement

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