

HYPOLIPIDEMIC EFFECT OF AQUEOUS EXTRACTS OF SELECTED SPICES AND THEIR MIXTURE ON DIET-INDUCED HYPERCHOLESTEROLEMIA IN WISTAR RATS

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ABSTRACT

This study evaluated the effect of aqueous extracts of garlic, ginger, pepper and their mixture on diet-induced hypercholesterolemia in rats. Hypercholesterolemia was induced using a diet containing 1% cholesterol and 25% soybean oil. Thirty six rats of Wistar strain weighing 168±10g were randomly distributed into six groups of six rats each- Group 1: control rats fed on the standard chow, group 2 rats fed on hypercholesterolemic diet and water only, while groups 3, 4, 5 and 6 were rats fed the hypercholesterolemic diet and orally with 0.5 ml of 200 mg/kg body weight extracts each of garlic, ginger, pepper and their mixture respectively for four weeks. At the end of 4 weeks, various biochemical indices as appropriate in the blood, liver and faeces of the rats were investigated. Significant reductions ($P<0.05$) in the weights, high organ/body weight ratio, atherogenic index (AI) and significantly ($P<0.05$) elevated lipid profile in the serum of hypercholesterolemic rats compared with the control rats was observed. Weights of rats treated with any of the spices or their mixture was significantly ($P<0.05$) elevated, increased fecal output of cholesterol and triglycerides in the last three days of the experiment in all the treated rats also indicated that administration of the spice extracts especially of the spice mixture led to significant ($P<0.05$) increased excretion of lipids. Use of these spices in the diet especially as a mixture, may therefore, ameliorate or prevent hypercholesterolemia in rats.

Keywords: Diet-hypercholesterolemia, lipid profile, mixture, spices, synergistic.

INTRODUCTION

Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It is a major risk factor in the development and progression of atherosclerosis that leads to cardiovascular diseases (Rerkasan *et al.*, 2008). Hypercholesterolemia poses a major problem to many societies especially the health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities (Ramachandran *et al.*, 2003; Matos *et al.*, 2005). Dietary factors such as continuous ingestion of high amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis (Asashina *et al.*, 2005). Clinical trials have demonstrated that intensive reduction of plasma low density lipoprotein (LDL-C) levels could reverse atherosclerosis and decrease the incidence of cardiovascular diseases (Ichihashi *et al.*, 1998).

Several spices have long been recognized to possess medicinal properties such as carminative, stomachic, antispasmodic, salt and sugar reduction, antihelmintic,

aphrodisiac, antinausea, lowering the risk of cardiovascular diseases, diabetes, antibacterial and anticancer agents (Rahman and Lowe, 2006; Kaur and Arora, 2009). Although these observations are largely empirical, the efficacious attributes have led to their pharmacological applications in the indigenous system of medicine all over the world. The active phytochemicals derived from these spices have also provided a molecular basis for these actions.

According to the World Health Organization, more than 65% of the world population still rely on traditional medicine for their health care needs (Peter, 2004; WHO, 2010). In Africa for instance, 80% of the population still depend on herbal medicine, while 65% of India's population still rely on Aryurvedic medical practices (Aschwaden, 2001). Spices have been reported to have ameliorative effect on hypercholesterolemia and therefore on cardiovascular diseases. Several *in vitro* studies have confirmed the ability of many spices especially garlic, ginger and pepper to reduce the risk factors associated with cardiovascular diseases (Alizadeh-Navaei *et al.*, 2008; Nicoll and Henein, 2009; Al Mofleh, 2010). The primary objective of this study therefore, is to biochemically evaluate the effect of garlic (*Allium sativum* L.), ginger (*Zingiber officinale* Roscoe), cayenne

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pepper (*Capsicum frutescens* L.) and their mixture on diet-induced hypercholesterolemia in Wistar rats.

MATERIALS AND METHODS

Materials

Casein, Cholesterol, and the assay kits for cholesterol, HDL-C, LDL-C and triglycerides are products of Randox Laboratories Ltd., Ardmore, Co. Antrim, UK. All other reagents used in this study were of analytical grade.

Animals and Diets

Thirty six (36) albino rats of Wistar strain weighing 168 ± 10 g were obtained from the Agricultural and Rural Development Research Institute, University of Fort Hare, South Africa. They were housed in clean Plexiglas cages with solid bottom in groups of six rats per cage, under controlled environment of temperature ($23 \pm 1^\circ\text{C}$), relative humidity ($55 \pm 10\%$) and 12 h light and 12 h dark cycle. Two experimental diets made up of the Control- standard rat chow, and the hypercholesterolemic which consisted of the control diet but with the inclusion of 1% cholesterol, 25% soybean oil, sucrose and cellulose (Matos *et al.*, 2005). Water and feed were made available ad libitum. Clearance was obtained from the ethics committee of the University of Fort Hare, Alice, South Africa. The animals were weighed at the beginning of the experiment and subsequently every week and their weights were recorded.

Preparation of spice extracts

The spices were individually sorted to remove grits and dirt, washed and thinly sliced. They were dried in the oven at 60°C for 72 h. The dried spices were milled into fine powder, packed into airtight plastic bottles and stored in the fridge at 4°C until needed. From the powdered samples, 50 g of each spice was mixed with 1000 ml of distilled water and boiled for 10 min at 100°C . It was allowed to cool, filtered and then freeze-dried (Vir Tis bench top K, Vir Tis Co. Gardiner, NY) for 48 h. The freeze-dried samples were stored in airtight plastic bottles under refrigeration (4°C) and reconstituted as needed for the various analysis.

To prepare the mixture of the spices, equal amounts of each spice [(50:50:50 g), (w/w/w)] were weighed and thoroughly mixed together by passing through a coffee grinder of a home blender set. From the mixture, 50 g of each spice was mixed with 1000 ml of distilled water and boiled for 10 min at 100°C . It was allowed to cool, filtered and then freeze-dried (Vir Tis bench top K, Vir Tis Co. Gardiner, NY) for 48 h. This mixture was stored in an airtight plastic bottle under refrigeration and reconstituted as needed for the various analyses.

Experimental procedure

Hypercholesterolemia was induced by feeding the rats on the diet high in cholesterol that has been established to induce hypercholesterolemia in a previous study (Matos *et al.*, 2005; Otunola *et al.*, 2010a) and 200mg/kg of spice extracts for four weeks. The rats were randomly distributed into six groups and each group fed appropriately- Group 1 consisted of control rats fed on the standard rat chow, group 2 rats were fed on hypercholesterolemic diet and water only, while groups 3, 4, 5 and 6 consisted of rats fed the hypercholesterolemic diet and orally gavaged with a 0.5 ml of 200 mg/kg body weight extracts each of garlic, ginger, pepper and their mixture respectively for four (4) weeks. The body weight and general conditions of the animals were monitored weekly, while feed and water intake were determined daily. Faeces were collected the first three and last three days of the experiment, (weighed and frozen until needed for analysis) to monitor the uptake and excretion of cholesterol and triglyceride. At the end of 4 weeks, all the animals were fasted overnight, anaesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg, i.p). Blood samples were collected by cardiac puncture into vacutainers (BD Plymouth, PL67BP, UK) for the various haematological and biochemical analyses.

Biochemical analyses

Lipid analysis

Serum total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG), were estimated by automatic analyzer techniques using the BECKMAN COULTER SYNCHRON[®] CLINICAL SYSTEMS and UniCel[®] Dx C 600/800 systems.

Atherogenic index (AI) was calculated as described by Lee and Niemann (1996) using the equation:

$$\text{Atherogenic Index (AI)} = \frac{\text{Total cholesterol} - \text{HDL-C}}{\text{HDL-C}}$$

Liver and fecal cholesterol and triglycerides were extracted with chloroform: methanol mixture (2:1; v/v) Folch *et al.* (1957) and were measured as described by Carr *et al.* (1993) using Randox kits.

Statistical analysis

The data from all the analyses were collected and statistically analyzed and expressed as the mean \pm SD ($n=6$), for each group of rats using one-way analysis of variance (ANOVA). SAS (2002) statistical tool was used to analyze the data obtained. Results were considered statistically significant at $P < 0.05$.

RESULTS

Feed intake and growth response of the hypercholesterolemic rats

Figure 1 shows the feed intake patterns of the rats for the duration of the experiment. Feed intake of the control rats were significantly ($P < 0.05$) higher than for the experimental rats, increasing from the first week through to the end of the experiment. The feed intake of the hypercholesterolemic rats dropped significantly ($P < 0.05$) right from the start of the experiment to the end. The hypercholesterolemic rats fed with spice extracts

displayed a gradual increase in feed intake, with HC rats treated with the mixture extract displaying a significantly ($P < 0.05$) higher intake amongst the treated rats. This group was followed by rats on the garlic, ginger and pepper treatments respectively.

Water intake (Fig. 2) of all the rats both control and experimental rats did not follow any particular pattern. It is however noteworthy that all experimental groups had the highest water intake on weeks 1 and 2. However, those treated with the ginger and pepper extracts had low water intake during this period.

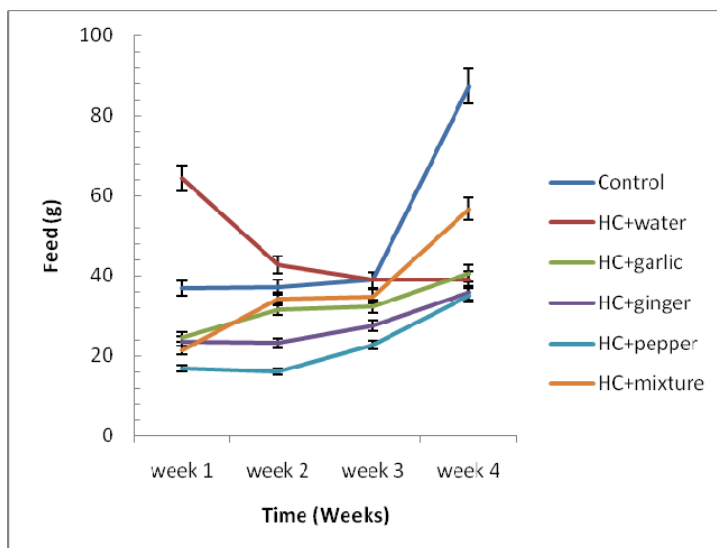


Fig. 1. Feed intake of Hypercholesterolemic rats treated with extracts of garlic, ginger, pepper and their mixture over a period of four weeks. Values are means of 6 determinations \pm SEM.

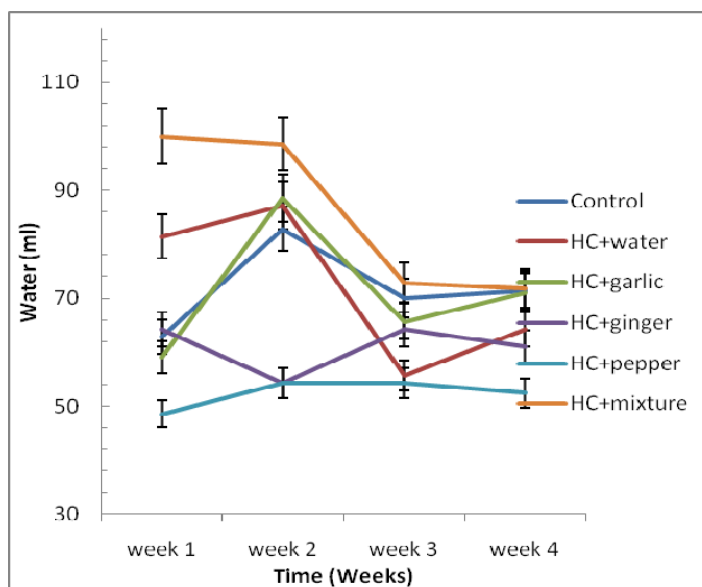


Fig. 2. Water intake of Hypercholesterolemic rats placed on aqueous extracts of garlic, ginger, pepper and their mixture over a period of four weeks. Values are means of 6 determinations \pm SEM.

Figure 3 displays the weight increase of the experimental rats. During the four weeks, the control rats showed a steady increase in weight compared with the hypercholesterolemic rats. Rats placed on the HC diet and water alone consistently lost weight throughout the period of the experiment. Hypercholesterolemic rats treated with the spice extracts also lost weight significantly ($P < 0.05$) by the end of the first week, but started gradual weight increase by the second week through to the end of the experiment.

The percentage organs to body weight ratio of the Hypercholesterolemic rats are shown in table 1. The liver weight was significantly ($P < 0.05$) higher in the hypercholesterolemic rats compared with the control and the spice treated rats. The liver, heart, kidney and brain ratio decreased significantly in all the spice treated groups compared to the hypercholesterolemic group.

In addition, there was no significant difference ($P > 0.05$) in the heart and kidney weights of the spice treated hypercholesterolemic rats when compared with the

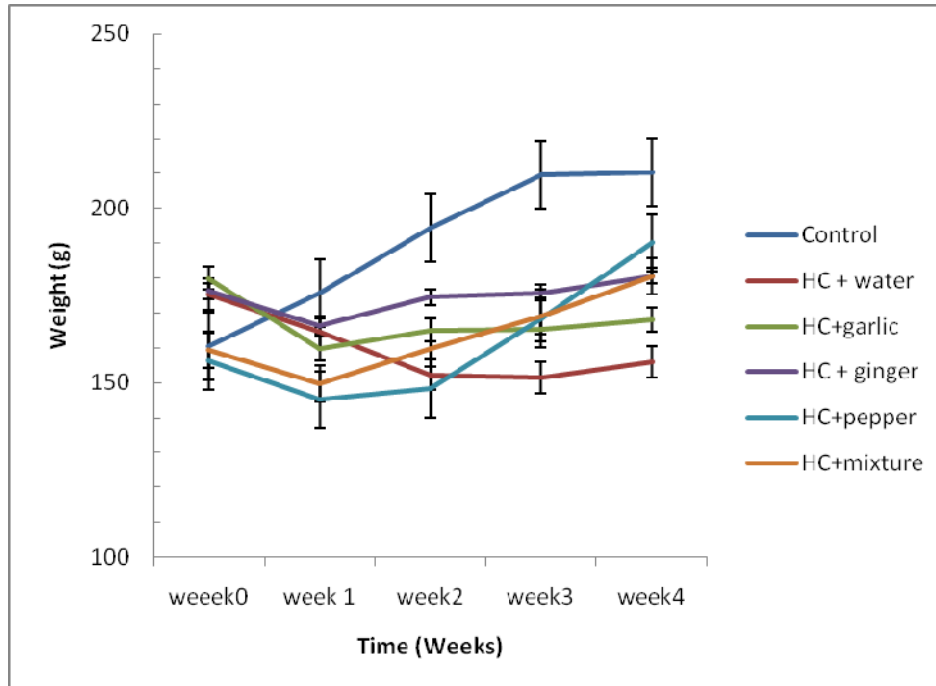


Fig. 3. Growth response of Hypercholesterolemic rats treated with extracts of garlic, ginger, pepper and their mixture. Values are means of 6 determinations \pm SEM.

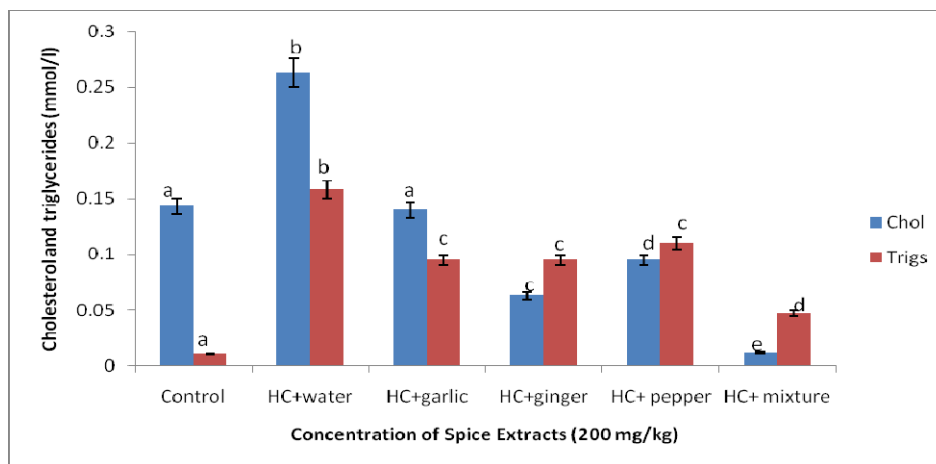


Fig. 4. Hepatic cholesterol and triglycerides concentrations of the hypercholesterolemic rats treated with spice extracts over a period of four weeks. Values are means of 6 determinations \pm SEM. Bars with different superscripts are significantly different ($P < 0.05$).

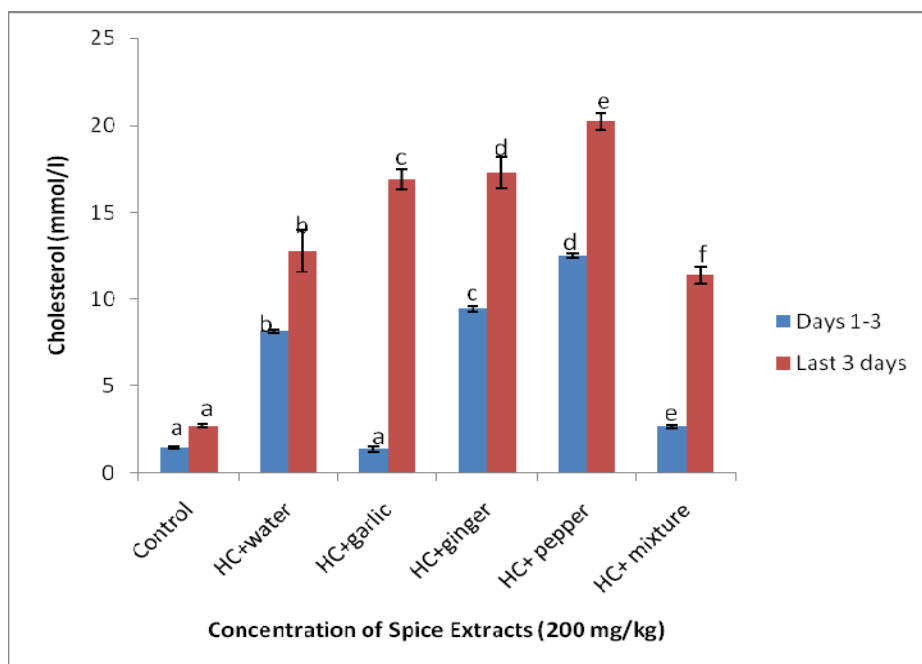


Fig. 5. Feecal cholesterol concentrations of the hypercholesterolemic rats treated with garlic, ginger, pepper and their mixture. Values are means of 6 determinations \pm SEM. Bars with different superscripts are significantly different ($P<0.05$).

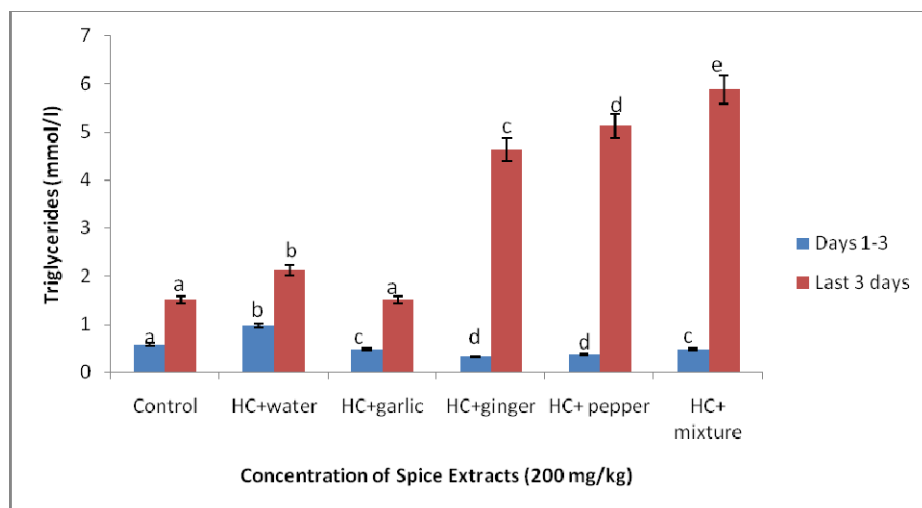


Fig. 6. Feecal triglycerides concentrations of the hypercholesterolemic rats treated with garlic, ginger, pepper and their mixture. Values are means of 6 determinations \pm SEM. Bars with different superscripts are significantly different ($P<0.05$).

control. The weight of the brain of the untreated hypercholesterolemic rats was higher than that of the control rats and those placed on the garlic, ginger and mixture extracts, however, the hypercholesterolemic rats on the pepper extract showed a significantly higher brain/body weight than all the experimental animals.

The serum lipid profile for the rats is shown in table 2. Total cholesterol, triglycerides, LDL-C and atherogenic index (AI) were significantly ($P<0.05$) high in the rats fed

with the hypercholesterolemic diet and water alone increasing at 1.93, 3.04, 30 and 119- times respectively over control values, while the HDL-C level decreased by 1.71-times compared with control values. Among the hypercholesterolemic rats treated with spices, the total cholesterol and triglyceride values were not significantly different ($P> 0.05$) from one another, but different from the control and from the untreated. Administration of the spice extracts led to a significant ($P<0.05$) reduction in the LDL-C and AI, while there was an increase in the HDL-C

of the treated rats especially with garlic and ginger extracts when compared with the untreated rats.

The hepatic lipids, figure 4, were significantly higher ($P < 0.05$) in untreated hypercholesterolemic rats compared with the control rats. Hepatic total cholesterol and triglyceride concentrations showed 1.84- and 14.36- times increases respectively in the hypercholesterolemic rats compared to the control rats.

In contrast, hypercholesterolemic rats treated with 200 mg/kg body weight boiled aqueous extracts of garlic, ginger, pepper or their mixture, had a significant ($P < 0.05$) decrease in the cholesterol and triglycerides concentrations. This decrease was most evident with those treated with the mixture extract which greatly reduced the hepatic lipids as a result of synergistic effect of combining the spices.

Total fecal cholesterol and triglyceride concentrations of the experimental rats are shown in figures 5 and 6 respectively for the first and last 3 days. In all the treatments, both fecal cholesterol and triglycerides increased significantly ($P < 0.05$) in concentration in the last 3 days over the first three days. Administration of the spice extracts increased the rate of fecal excretion for both cholesterol and triglycerides in hypercholesterolemic rats. The animals that were treated with 200 mg/kg body

weight of pepper extract showed the highest fecal excretion of cholesterol, while the highest fecal excretion of triglycerides was shown by hypercholesterolemic rats treated with the mixture of spices. Fecal excretion of both cholesterol and triglycerides was significantly ($P < 0.05$) lower in untreated hypercholesterolemic rats.

DISCUSSION

It is long known that there is a relationship between the high prevalence of cardiovascular diseases and abnormalities in lipid metabolism. Also, a positive correlation between dietary fat, hypercholesterolemia, hyperlipidemia and the incidence of coronary diseases has been established and documented (Harnafi *et al.*, 2009). Previous evidence suggests that modification of diet composition using plant foods and spices have successfully prevented hyperlipidemia and atherosclerosis (Aguilera *et al.*, 2003; Jung and Wang, 2009).

In the current study, hypercholesterolemia-induced diet feeding for four weeks was chosen as the experimental model of early phase atherogenesis, while the role of the boiled aqueous extract of garlic, ginger, cayenne pepper and their mixture in countering the lipidemic-oxidative aberrations that comes with diet-induced hypercholesterolemia have also been investigated.

Table 1. Percentage (%) organ to body weight ratio of hypercholesterolemic rats treated with extracts of garlic, ginger, pepper and mixture.

Group	Liver	Heart	Kidney	Brain
Control	3.28±1.37 ^a	0.36±0.02 ^a	0.7±0.07 ^a	1.18±0.16 ^a
HC+water	4.18±1.09 ^b	0.34±0.01 ^a	0.82±0.10 ^b	1.62±0.10 ^b
HC+garlic	3.78±1.41 ^{ac}	0.36±0.02 ^a	0.63±0.07 ^a	1.00±0.02 ^a
HC+ginger	3.30±0.98 ^a	0.43±0.02 ^{ab}	0.86±0.07 ^b	1.47±0.08 ^b
HC+pepper	3.90±1.95 ^{ac}	0.40±0.01 ^{ab}	0.82±0.08 ^b	2.10±0.27 ^c
HC+mixture	3.25±1.00 ^a	0.35±0.02 ^a	0.82±0.02 ^b	1.44±0.18 ^b

Values are means of 6 determinations ± SEM. Values along the same column with different superscripts are significantly ($P < 0.05$) different. HC= Hypercholesterolemic rats.

Table 2. Serum lipid profile of hypercholesterolemic rats treated with garlic, ginger pepper and mixture (mmol/l).

Group	TC	TG	HDL-C	LDL-C	AI
Control	1.28 ± 0.12 ^a	0.45±0.36 ^b	1.25±0.16 ^c	0.01±0.00 ^a	0.02
HC+ water	2.47 ± 0.14 ^b	1.37±0.16 ^a	0.73±0.10 ^b	0.30±0.00 ^b	2.38
HC+ garlic	1.83 ± 0.4 ^c	0.50±0.15 ^b	1.63±0.27 ^a	0.07±0.00 ^b	0.12
HC+ ginger	1.53 ± 0.16 ^d	0.40±0.08 ^b	1.37±0.19 ^c	0.07±0.10 ^b	0.12
HC+ pepper	1.33 ± 0.05 ^d	0.53±0.08 ^b	1.13±0.08 ^c	0.01±0.00 ^a	0.18
HC+ mixture	1.36 ± 1.21 ^d	0.30±0.11 ^b	1.00±0.43 ^c	0.03±0.68 ^b	0.90

Values are means of 6 determinations ± SEM. Values along the same column with different superscripts are significantly ($P < 0.05$) different. HC- hypercholesterolemic diet, TC-total cholesterol, TG-triglycerides, HDL-C- high density lipoprotein cholesterol, LDL-C- low density lipoprotein cholesterol, AI- atherogenic index.

It was observed that the high fat content of the diet led to its lower ingestion by the rats on the hypercholesterolemic diet, consequently leading to a lower ingestion of other nutrients and weight loss in the untreated hypercholesterolemic animals compared with the control and the hypercholesterolemic rats treated with the spice extracts. However, administration of a 200 mg/kg body weight of the boiled aqueous extract of any of garlic, ginger, pepper or their mixture along with the hypercholesterolemic diet led to improved feed intake and subsequent weight gain in the treated rats. The significant increase in the weight of the liver relative to the body weight of the hypercholesterolemic rats compared with the control could be a consequence of accumulation of liver lipids, even as there was a downward decrease in body weight. The loss in weight could be as a result of insufficient nutrient intake caused by low feed intake because, according to Dutta *et al.* (2008), feed intake is lowered when a diet is high in fat leading to reduced nutrient intake. This trend agrees with previous observations when rats were induced with hypercholesterolemic diets (Ogunola *et al.*, 2010a). The normalization in the feed intake, weight gain and decreased liver/body weight of the hypercholesterolemic rats treated with extracts of the spices could be as a result of the ameliorative effect of the spices leading to increased appetite and inhibition of accumulation of hepatic lipids in the rats treated with the spice extracts. These findings agree with the reports of Khalil *et al.* (2010); Bujanda *et al.* (2008); Kwon *et al.* (2005) that *Citrullus colocynthis*, resveratrol and traditional Chinese medicine respectively possessed a similar effect on the liver weight.

The results of this study demonstrates that high-cholesterol/high-fat feeding in rats caused significant increases in the level of circulating cholesterol, triglycerides, LDL-C and atherogenic index in hypercholesterolemic rats, while the HDL-C decreased significantly. Elevation of these parameters could lead to atherosclerosis. These observations are consistent with many reported studies (Prasad, 2005; Park *et al.*, 2009; Pyo and Seong, 2009; Al-Attar, 2010). Excessive dietary intake of fat causes a rise in serum cholesterol by down-regulating the LDL-C receptor synthesis leading to a reduction in the uptake of LDL-C by the receptor, which results in an increase in cholesterol level (Al-Attar, 2010). According to the National Cholesterol Education Programme (NCEP, 2001) guidelines, lowering LDL-C is the primary objective of coronary heart disease reduction, hence, there is an increasing demand for a medical treatment for this problem.

Administration of 200 mg/kg body weight of the boiled aqueous extracts of garlic, ginger, pepper or their mixture for four weeks was effective in significantly improving the serum lipid profile by lowering total cholesterol,

triglyceride, LDL-C and atherogenic index in rats fed on hypercholesterolemic diets compared with the control. Also, these extracts could reduce the risk of atherosclerosis because the HDL-C was significantly higher in the spice-treated rats. Moreover, the atherogenic index in the treated group was greatly reduced compared with the hypercholesterolemic group. Atherogenic index (AI) indicates the risk for the deposition of foam cells, plaque, fatty infiltration or lipids in heart, coronaries, aorta, liver and kidney (Basu *et al.*, 2007). The higher the AI, the higher the risk of oxidative damage in these organs. These hypolipidemic and anti-atherogenic properties of the extracts of garlic, ginger, pepper and their mixture, are probably due to the presence of antioxidant compounds such as flavonoids, phenolics, proanthocyanidin and flavonols in the spices. This is in agreement with the findings reported by Rehrah *et al.* (2007); Yang and Koo (1997) who studied the benefits of some green teas on fatty livers induced by high cholesterol feeding.

In addition, each of the extracts of garlic, ginger and pepper has been reported to significantly counter hypercholesterolemia predominantly reducing the LDL-C fraction in serum and liver, increase fecal excretion of cholesterol and triglycerides, reduce atherogenic index and enhance energy metabolism (Srinivasan, 2005; Tattleman, 2005; Amagase, 2006; Manjunatha and Srinivasan, 2008).

Available information in literature has not revealed the use of a mixture of garlic, ginger and pepper in the treatment of hypercholesterolemia. It is exciting to report here that a 200 mg/kg body weight dose of the boiled aqueous extract of a mixture of the three spices brought about a significant decrease in serum and hepatic total cholesterol, triglycerides, LDL-C and atherogenic index levels even down to control levels. Also the rise in HDL-C was higher in groups given the extract of this mixture. This is believed to be as a result of additive/synergistic action of the three spices which individually exhibited lipid lowering effects. This is similar to the report of Shobana and Naidu, (2000) who reported that a spice mix of (ginger, onion and garlic; onion and ginger; ginger and garlic) showed a cumulative inhibition of lipid peroxidation by exhibiting a synergistic property when compared with the individual spices. Seah *et al.* (2010) also reported a similar trend with keang-hleung paste (a mixture of turmeric rhizome, garlic and chili).

CONCLUSION

The findings of the present study suggests that the administration of a 200 mg/kg body weight aqueous extract of either garlic, ginger, pepper or their mixture along with hypercholesterolemic diet, led to improved weight gain in male Wistar rats. It also succeeded in

lowering serum total cholesterol, triglycerides, LDL-C and atherogenic index, while elevating serum HDL-C.

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