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Effect of Aqueous Extract of *Piper nigrum and Zingiber officinale on the* Serum Cholesterol and Triacylglycerol Concentrations of Albino Rats

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ABSTRACT
The effects of aqueous extracts of *Piper nigrum* fruits and *Zingiber officinale* rhizomes on the serum cholesterol and triacylglycerol concentrations of rats were investigated. Phytochemical analyses were also carried out on the extracts. Sixteen male rats, randomly distributed into 4 groups (A-D) of 4 rats each, were orally administered with the extracts daily for 21days. The groups A-C animals were administered with *P. nigrum, Z. officinale* and *P. nigrum* + *Z. officinale* at 400 mg/kg body weights respectively, while group D served as the control. Flavonoids, saponins, terpenes and steroids were found present in both plants with alkaloids not present in any of them. The results showed that the aqueous extracts of both plants significantly ($p < 0.05$) reduced the serum cholesterol and triacylglycerol concentrations of the animals. A 1:1 mixture of the plants' extracts significantly ($p < 0.05$) reduced the concentrations of these serum lipids more than individual treatments using the plants. These findings indicate that the use of extracts of both plants together synergistically enhanced their serum lipid lowering potentials. The findings could be of nutritional and clinical importance to individuals at risk of cardiovascular disease.

Keywords: Black pepper, Ginger, Hypocholesterolemia, Hypotriglyceridemia

INTRODUCTION

Spices have been used for thousands of years by many cultures around the globe to enhance flavour, aroma and presentation of foods as well as for their medicinal values (Zaika, 1988). They vary from leafy vegetables, roots, fruits to seeds. *Piper nigrum* and *Zingiber officinale* are among the commonly and widely used spices.

P. nigrum (black pepper), which belongs to the family Piperaceae, is a smooth woody vine that can grow up to 33 feet in hot humid tropical climates. It begins to bear small white clustered flowers after 3 to 4 years of planting and these develop into berries known as pepper corns. Ground pepper corns produce the pungent and aromatic spice called pepper. The pungency is strongest in white and weakest in green pepper. *P. nigrum* is a good source of manganese, iron and dietary fiber with demonstrated impressive antioxidant and antibacterial effects (Dorman and Deans, 2000). It has long been recognized to have carminative (prevents formation of intestinal gas), diaphoretic (promotes sweating) and diuretic (promotes urination) properties (Murray, 1993).

Z. officinale Roscoe (ginger) is a perennial herb that belongs to the Zingiberaceae family. The plant produces an orchid-like flower with petals that are greenish-yellow streaked with purple colour. Ginger is a native of southern Asia and is cultivated in areas of China, Jamaica, Nigeria and Sierra Leone, among others. Ginger rhizome has been found to be a good source of nutrients, minerals such as magnesium, iron, calcium and phosphorous as well as vitamins including thiamine, riboflavin, niacin and vitamin C (Govindarajan, 1982). It owes its pungency and aroma to gingerol and volatile oils respectively, present in the rhizome. Ginger is an essential ingredient in many traditional Chinese medicines and has been used since 4th century BC. It has been reported to posses carminative, diaphoretic, antispasmodic, expectorant, astringent, antioxidant, anti-inflammatory and diuretic properties as well as peripheral circulatory, appetite and digestive

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acid stimulant effects (Rockville, 1998). It is also used traditionally to relieve and prevent nausea caused by motion and morning sickness.

Herbal remedies are becoming increasingly popular and scientific evidence of efficacy of these herbs is beginning to emerge from controlled pre-clinical and clinical trials. And of course, a good number of commonly used pharmaceutical products are of botanical origin. Artemisinin, aspirin, quinine, digitoxin and reserpine are well known examples (Acshwanden, 2001). Some of these herbal products have found usage in the management of various ailments including lowering of certain blood markers of disease. For instance, extracts of *Spondias mombin* leaf have been found to have serum lipid lowering effects (Igwe *et al*, 2008). Furthermore, it has been reported that lowering of the concentrations of plasma lipids could diminish the complications of atherosclerosis, thus preventing cardiovascular disease and thereby prolonging life (Brown and Goldstein, 1992; Ostland, 2002).

The aim of the present study was to investigate the effects of the crude aqueous extracts of *P. nigrum* and *Z. officinale* on serum cholesterol and triacylglycerol concentrations since the blood concentrations of lipids tend to correlate positively with increased prevalence of cardiovascular diseases (Cromwell and Otvos, 2004).

MATERIALS AND METHODS

Plant materials

Fresh *P. nigrum* fruits and *Z. officinale* rhizomes were obtained from Feringada market in Jos, Plateau State. They were identified at the Department of Botany, University of Jos, Nigeria.

Phytochemical analysis

The phytochemical analysis of the plant samples for the presence of flavonoids, saponins, tannins, alkaloids, anthraquinones and cardiac glycosides were carried out according to the methods described by Harborne (1973) and Trease and Evans (1989). Terpenes and steroids presence were identified using Liebermann Burchard and Salkowski tests respectively, as described by AOAC (1990).

Extraction procedures

The plant samples were washed and dried in an oven at 40^0C to a constant weight. Two hundred grams (200 g) of the dried plant samples were peeled, ground into fine powder and extracted with 500 ml of distilled water at room temperature for 72 hours. The extract was then boiled for 45 minutes, filtered through cheese-cloth and Whatman No. 1 filter paper. The filtrate obtained was evaporated to dryness in an oven and stored in screw-top containers in a refrigerator until required for use.

Animal experimentation

Sixteen healthy adult male albino rats weighing between 190 and 250 g were obtained from National Veterinary Research Institute Vom, Plateau State. The animals were kept in the Department of Biochemistry Laboratory for one-week to allow for acclimatization. They later re-weighed and divided into four groups (A-D) of four rats each according to their body weights. They were maintained on standard diet of growers mash (Grand Cereal and Oil Mills Limited, Nigeria) and water *ad libitum.* The test groups (A-C) were administered daily doses of ginger extract, black pepper extract and a 1:1 mixture of ginger and black pepper extracts respectively at 400mg of extract per kg body weight of animals. The last group (D) animals were used as the control group and were not administered any extract. All administrations were orally for a period of twenty-one days.

Preparation of serum

At the end of the experimental period, 5-8ml of blood samples were collected from each animal by cardiac puncture and dispensed into labeled plain containers. After clotting and retracting, the blood samples were centrifuged at 3,000 rpm and the supernatant sera samples separated and used for cholesterol and triacylglycerol determinations.

Determination of serum cholesterol concentration

The quantitative method of Richmond (1973) was used to determine the serum total cholesterol concentrations of the samples using diagnostic kits supplied by Biosystems Ltd, USA.

Determination of serum triacylglycerol concentration

The serum triacylglycerol concentrations of sera samples were determined according to the method of Bucalo and David (1973).

Statistical analysis

Statistical analysis of data obtained was carried out using ANOVA and Duncan's New Multiple Range test. Significant differences were accepted at $p < 0.05$.

RESULTS

Phytochemical screening of the plant samples showed the presence of flavonoids, saponins, terpenes and steroids, while alkaloids were not detected in both spices. Curiously cardiac glycosides and anthraquinones were found present in only the *Z. officinale* rhizome (Table 1).

Crude aqueous extract of *P. nigrum* fruit did not have any significant ($p > 0.05$) reducing effect on the serum cholesterol concentrations (Table 2). On the other hand, extracts of *Z. officinale* rhizome only, and in combination with *P. nigrum* caused significant ($p < 0.05$) reductions in the cholesterol concentrations of the test animals when compared to corresponding values obtained for the control animals. Serum triacylglycerol concentrations were significantly ($p < 0.05$) reduced in the animals after treatment with the extracts of the spices, with the highest effect observed among animals treated with a mixture of both spices.

Table 1: Phytochemical profiles of *P. nigrum* and *Z. officinale*

Phytochemical	***P. nigrum***	***Z. officinale***
Flavonoids	+	+
Saponins	+	+
Tannins	+	-
Alkaloids	-	-
Anthraquinones	-	+
Cardiac glycosides	-	++
Terpenes	+	++
Steroids	+	+++

Key: + = Detected; - = Not detected

Table 2: Effects of aqueous extracts of *P. nigrum* and *Z. officinale* on serum cholesterol and triacylglycerol concentrations.

Parameters	**Groups**			
	A	**B**	**C**	**D**
Cholesterol (mmol/l)	10.51 ± 1.53^{ac}	9.92 ± 1.23^{a}	6.95 ± 1.74^{b}	12.13 ± 0.11^{c}
Triacylglycerol (mmol/l)	0.65 ± 0.20^{ab}	0.83 ± 0.10^{a}	0.53 ± 0.10^{b}	1.08 ± 0.19^{c}

Keys: A = *P. nigrum* group; B = *Z. officinale* group; C = *P. nigrum*+*Z. officinale* group; D = Control group. Values are mean ± standard deviations of triplicate determinations. Values with different superscripts per row are statistically significant ($p < 0.05$).

DISCUSSION

The medicinal use of plants' parts in the management and treatment of diseases have been an age long practice. The continued investigation into the secondary plant metabolites has led to important breakthroughs in pharmacology. This has also helped in the development of modern pharmacotherapeutics in Africa and other parts of the world (Nwaogu *et al.,* 2007). Thus, plants have continued to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products (Sofowara, 1982). These secondary plant metabolites exhibit varied biochemical and pharmacological actions in animals when ingested (Amadi *et al.,* 2006). Thus flavonoids have been associated with antioxidant, diuretic, antispasmodic and anti-inflammatory activities (Owoyele *et al.,* 2002), while tannins and flavonoids have also been reported to have antimicrobial effects (Abo *et al.,* 1999). Furthermore, phytochemicals such as the saponins, polyphenols, anthraquinones, alkaloids, tannins and cardiac glycosides are biologically properties. Saponins are known anti-nutritional factors, which reduce the uptake of certain nutrients including glucose and lipids especially cholesterol at the gut through intra-lumenal physicochemical interaction. Hence, saponins have been reported to have hypocholesterolemic effects (Price *et al.,* 1987). The presence of saponins in both *P. nigrum* and *Z. officinale* may account for the significant reduction in serum cholesterol and triacylglycerol concentrations observed in this study, especially when a combined mixture of both plants' extract is used for treatment.

Cholesterol, triacylglycerol and fatty acids are significant and independent risk factors of adverse cardiovascular events (Wierzbicki and Mikhailidis, 2002). The results of the present study showed that consumption of both *P. nigrum* and *Z. officinale* can help in reducing the risk of hyperlipidemia and atherosclerosis. The higher effect observed with a mixture of both spices indicates synergistic effect occasioned by the mixture of the medicinally active phytochemicals present in the two plants.

In conclusion, aqueous extracts of *P. nigrum* and *Z. officinale* have blood cholesterol and triacylglycerol lowering effect, especially when taken together, which may be of clinical benefit to individuals at risk of cardiovascular disease. Considering the reported reduction in complications of cardiovascular diseases with lowering of plasma lipids concentrations, the use of these spices especially in resource-poor economies by an impoverished populace should be strongly promoted. There may also be need to encourage commercially spice manufacturers to incorporate these important spices in their menu.

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