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Piper species protect cardiac, hepatic and renal antioxidant status of atherogenic diet fed hamsters

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ABSTRACT

Pre-clinical and clinical studies points to the use of antioxidants as an effective measure to reduce the progression of oxidative stress related disorders. The present study evaluate the effect of three *Piper* species (*Piper guineense*, *Piper nigrum* and *Piper umbellatum*) for the protection of cardiac, hepatic and renal antioxidant status of atherogenic diet fed hamsters. Hamsters were classified into eight groups: a normal control, atherogenic control and six other experimental groups (fed atherogenic diet supplemented with different doses of *P. nigrum*, *P. guineense* and *P. umbellatum* (1 and 0.25 g/kg) for 12 weeks. At the end of the feeding period the heart, liver and kidney from each group were analyzed for lipid profile and antioxidant enzymes activities. Atherogenic diet induced a significant ($P < 0.001$) increase in the lipid profile across the board and equally significantly altered the antioxidant enzyme activities. Supplementation with *Piper* species significantly inhibited the alteration effect of atherogenic diet on the lipid profile and antioxidant enzymes activities. The *Piper* extracts may possess an antioxidant protective role against atherogenic diet induced oxidative stress in cardiac, hepatic and renal tissues.

1. Introduction

High fat diet (atherogenic diet) is commonly used to induce atherosclerosis (Agbor, Oben, Ngogang, Xinxing, & Vinson, 2005; Nicolosi, 1991; Vinson et al., 2006), oxidative stress (Agbor, Vinson, Patel, et al., 2007; Agbor, Vinson, Sortino, & Johnson, 2010), hyperlipemia and obesity (Belguith-Hadriche et al., 2010; Decorde, Teissedre, & Sutra, 2009; Hansen, Han, Nolte, Chen, & Holloszy, 1997) in rodent animal models. These are all similar pathogenesis found in humans when intoxicated with high fat diet. Three organs highly exposed to oxidative stress are the heart (based on circulation), the liver (the site of metabolism) and the kidney (site for excretion). These organs are often studied in order to access the degree of oxidative stress/damage in animals (Belguith-Hadriche et al., 2010; Decorde et al., 2009; Deepa & Varalakshmi, 2003, 2004; Kumar, Sudhahar, & Varalakshmi, 2005; Tokuno, Thorèn, Löwbeer, & Valen, 2001). These organs have a self protective antioxidant enzymes system (superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase) that could help reduce oxidative stress. However, intoxication with high fat diet has been reported to overcome this defense mechanism and hence resulting in oxidative stress (Agbor, Vinson, Sortino, et al., 2010; Decorde et al., 2009; Deepa & Varalakshmi, 2003; Kempaiah & Srinivasan, 2002; Kumar et al., 2005). Increased oxidative stress, arising from high fat diet appears to play an important role in chronic inflammation responses to hypercholesterolemia atherosclerosis. Cardiac reactive oxygen species has been associated with atherosclerosis development (Heymes et al., 2003) while an atherogenic diet induces superoxide radical production (Riss et al., 2007) resulting in lipid peroxidation generating more peroxide radical.

Previous reports on *Piper* species showed that they are potent free radical scavengers, with strong antioxidant activity against copper induced lower density lipoprotein oxidation, hence inhibiting the formation of foam cells and development of atherosclerosis (Agbor et al., 2005; Agbor, Vinson, Oben, & Ngogang, 2007, 2010; Agbor, Vinson, Sortino, et al., 2010). *Piper* species are used as spices in most African diets and their seeds are referred to as the African black pepper or peppercorn. The present study test the hypothesis that *Piper nigrum*, *Piper guineense* and *Piper umbellatum* prevent the deleterious effect of atherogenic diet on hamsters as measured by antioxidant activities and lipid profiles of cardiac, hepatic and renal tissues. The objective of this study was to examine that *Piper* species protects the antioxidant status of cardiac, hepatic and renal tissues of atherogenic diet fed hamsters.

2. Materials and methods

The trial was run in accordance with the practice and principles of the institutional ethics review board of the University of Scranton (Institutional Animal Care and Use Committee (IACUC)), the

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1996 Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act that pertains to research of this nature. The animal protocol was approved by the IACUC of the University of Scranton, reference number # 2-07 of 11/07/07.

2.1. Plant material

Fresh leaves of the three herbs were harvested from their natural habitat in Yaoundé, Cameroon with the aid of an Ethnobotanist technician. The fresh leaves were cleaned with tape water and air-dried in the laboratory at room temperature. The dried plant material 50 g was then macerated in methanol for 48 h and filtered. The procedure was repeated twice with the residue and the filtrates were put together. The filtrate was then concentrated using a rotary evaporator with the aid of a vacuum pump. The concentrate was then converted into dry powdered material with the aid of a freeze dryer and stored at −20 °C until required. Same procedure was used for all three plant materials. The percentage yield of the extraction was 5.34% for *P. umbellatum*, 5.88% for *P. guineense*, and 9.27% for *P. umbellatum*.

2.2. Experimental design and feeding

Fifty-six weanling male Syrian Golden hamsters purchased from Charles River were used in this experiment. Experimental animals (hamsters) were acclimatized to the animal room temperature, humidity, and environment and feeding habit with normal diet for a period of 2 weeks prior to the study. The animals were then divided into eight groups (seven hamsters each). One group served as the normal control, another as the atherogenic control while the remaining six groups served as the test groups. The normal control animals were fed normal rodent chow, while the atherogenic control animals were fed the same rodent chow supplemented with 0.2% cholesterol and 10% coconut oil (atherogenic diet). The six test groups' animals were fed same diet as the atherogenic control but with additional supplementation of two graded doses (1 g and 0.25 g/kg body weight) of lyophilized plant extracts. The feeding process lasted for 12 weeks during which all the animals had regular supply of clean water. At the end of the experimental period, all the animals were fasted for 24 h. They were then anaesthetized. After the death of the animals, the organs (liver, heart and kidney) were collected rinsed in phosphate buffer saline (pH 7.4) and weighed. The different dose administration was adapted from earlier study (Agbor, Vinson, Sortino, et al., 2010).

2.3. Analysis of antioxidant enzymes

The organs were then homogenized in cold phosphate buffer (50 mM, pH 7.0). The solution was vortex for 2 min and then centrifuged at 8000 rpm for 20 min at 4 °C. The homogenates supernatants were analyzed for the activities of superoxide dismutase (SOD) (Sun, Oberly, & Li, 1988), catalase (CAT) (Beers & Sizer, 1952), glutathione reductase (GRX) (Carlberg & Mannervik, 1977) and total protein concentration (Bradford, 1976).

2.4. Analysis of membrane lipids

For the membrane lipids, half a gram of the organ was homogenized in an isopropanol–chloroform mixture (2:1) and centrifuged at 5000 rpm for 30 min. Then cholesterol (Searcy & Bergquist, 1960) and phospholipids (Connerty et al., 1960) were analyzed from the supernatant and cholesterol/phospholipid ratio calculated.

2.5. Statistical analysis

At the end of the experimentation, results were subjected to statistical analyzes. Analysis of Variance on Ranks was employed in groups' comparison. All pair wise multiple comparisons to determine significant difference between groups was determined using Holm–Sidak method. The SigmaStat (Systat software, Richmond, CA) version 3.01 was employed in these analyzes.

3. Results

3.1. Effect of Piper species on relative organ weight and % body weight gain

Table 1 shows the relative organs weight and % body weight gain of experimental animals. The body weight increased in all groups throughout the experiment with no significant ($P < 0.05$) differences in percentage weight gain between groups. There were equally no significances in the heart and kidney to body weight ratios across the groups. However, the liver to body weight ratio increased significantly in atherogenic diet fed hamsters compared with those fed normal diet. *Piper* extracts did not have any significant effect on these increases.

3.2. Effect of Piper species on food consumption

The effect of*P. guineense*, *P. nigrum* and *P. umbellatum* (*Piper* species) on food consumption is presented in Fig. 1. Generally, atherogenic diet increased food consumption as compared to the control group. When supplemented with *Piper* species the food consumption increased the more. With the exception of the group 3 animals supplemented with 1 g/kg *P. guineense* the other *Piper* extracts supplemented groups had higher food consumption.

3.3. Growth curve of hamsters during the course of the experiment

The growth curve of experimental animals is presented in Fig. 2. During the experimental period, all the animals increased in weight gain. Though the atherogenic diet control animals (Group 2) appeared to gain more weight, these increases were not significantly different between groups.

3.4. Effect of Piper species on tissue antioxidant enzyme activities

Table 2 shows the alteration effect of cardiac, hepatic and renal antioxidant enzyme (SOD, CAT and GRX) system of atherogenic diet fed hamsters and the protection rendered by *Piper* treatment. Compared to the control group (normal diet), the atherogenic diet induced an increase in cardiac and hepatic SOD, CAT and GRX activities. This increase was 8.4- (SOD), 2.7- (CAT) and 1.8- (GRX) fold for the cardiac tissue; 1.6- (SOD), 2.1- (CAT) and 1.9- (GRX) fold for the hepatic tissue. Supplemented with *Piper* extracts significantly ($P < 0.002$) prevented the increase in antioxidant enzyme induced by atherogenic diet. The prevention for the least effective extract was (*P. umbellatum*) 6- and 1-fold for the cardiac SOD and CAT, respectively. The prevention of the least effective extract (*P. umbellatum*) for the hepatic tissue was by 1.5 times for CAT. Meanwhile the most effective extract for the cardiac protection was *P. nigrum* (0.25 g/kg) and *P. guineense* (1 g/kg) for SOD and CAT, respectively; and for the hepatic protection it was *P. nigrum* (0.25 g/kg) for both SOD and CAT. Similarly a significant decrease was observed in the cardiac GRX activity of experimental groups supplemented with *Piper* extracts which was not observed in the hepatic GRX as opposed to the atherogenic control group. In the renal tissue the atherogenic diet equally induced an increase in the antioxidant enzymes with a significant increase in CAT and GRX activities. However supplementation with *Piper* extracts tends to normalize these effects. The activity of SOD was not altered significantly ($P > 0.05$). This result showed that the three *Piper* species