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Full Length Research Paper

Histophysiological and basal metabolic responses of albino rat, *Rattus norvegicus* (L) exposed to aqueous pepper extracts

Chukwu, L.O.

Aquatic Toxicology and Ecophysiology Laboratory, Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos. E-mail: obinnalcunilag@yahoo.com.

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The effect of oral ingestion of aqueous extracts of two species of pepper, *Capsicum annum var fastigatum* (Linn) and *Capsicum frutescens var minima* (Linn) on the basal metabolic rate and histophysiology of the gastrointestinal tract and liver of *Rattus norvegicus* (L) was investigated. Body weight data was recorded weekly during the period of investigation. The various species of pepper produced a decrease in basal metabolic rate as the concentrations of the extract increased. Gross and histopathological changes observed in the gastrointestinal tract and liver were demonstrated as cellular damage under light microscopy and weight loss. The implications of these findings are discussed.

Key Words: Histophysiology, metabolic rate, albino rat pepper extracts.

INTRODUCTION

Hot red pepper (*Capsicum*) is the most common species used in food throughout the world. Pepper is an important condiment commonly used when red and dry for spicing and culinary. The thin pungent bright types are used for spicing, while the staunt mild types are utilised as vegetables and salads. The pimentos (spanish paprikas) which possess large fruits with smooth pericarp are used in canning industry for preservation purposes. It not only induces the tastes and aroma in many household pots of soup, but also enhances the keeping quality of same (Sirsatsmil and Khanolkar, 1960).

For more than a century, capsaicin (8-methyl-N-vanilly-6-noneamide) active ingredient of pepper has captured the attention of many investigators, because its biting and burning properties suggest it could have physiological and pharmacological effect. Early studies demonstrated that peripheral administration of capsaicin produced a hypothermic effect which failed to produce detectable changes in the metabolic rate (Glinsukon et al., 1979). However, results from later investigations indicate that the ingestion of red pepper decreases appetite and subsequent protein and fat intake in Japanese females and energy intake in caucasian males (Yoshioka et al., 1999). The authors further suggested that this effect might be related to an increase in sympathetic nervous system activity in caucasian males. Further studies on the medicinal properties of red pepper indicate that it decreases the intensity of dyspeptic symptoms probably through a desensitization of gastric nociceptive fibres induced by its content of capsaicin (Bortolotti et al., 2002).

Previous studies by Nopanitaya and Nye (1974) on the cytotoxic activity of capsaicin on rat absorptive cells following intra-doudenal and intra-gastric administration showed morphological changes in some cells: mitochondrion's matrix was rarefied and the cristae was disorganised, a number of free ribosomes and lysosomes were increased, and there was dilation of endoplasmic reticulum. A reduction in the terminal web density of absorptive cells during administration of capsaicin has also been observed (Nopanitaya, 1973). These authors suggested that this might be as a result of damage to some of the filament materials within this region or to attenuation of some by changes in cell volume.

Pepper occupies a unique place in the life West Africans. They are not only consumables of everyday use in the social and religious life of the people, but also have an immense economic value. The dried fruit of pepper may be used locally as an irritant for rheumatism or neuritic as a gargle for throat inflammation and for alcoholic gastritis, and certain type of diarrhoea (Sundararaj and Thulasidasia, 1980). The high rate of pepper consumption has made it imperative that the effect of these plant extracts on the metabolic rate and general physiology of the mammalian gastro-intestinal tract should be investigated.

MATERIALS AND METHODS

Source of materials

Albino rats which were 5 – 6 weeks old with a mean weight of 83.65 ± 1.35 g, obtained from the Animal House, University of Lagos, were used for this study. The rats were all male to avoid any physiological differences that might arise due to sex. The pepper used were purchased from stalls and shops along the University of Lagos Road. Two different species of dried red pepper were bought: *Capsicum annum var fastigiatum* (Linn) (hot red pepper) and *Capsicum frutescens var minima* (Linn) (Cayenne pepper).

Preparation of pepper extracts

A mammalian physiological solution, the Tyrode Ringer Solution, was prepared following the method of Perry (1971). To obtain pepper extracts, the pepper were ground mechanically using mortar and pestle. This was done so that the plant tissue could be broken to release cellular contents which included the active ingredient. 10 g of each species of the ground pepper was dissolved in 50 ml of mammalian tyrode solution. The mixture was kept for 12 h at room temperature to allow the active ingredient in the pepper to diffuse in to the solution and the concentrated solution was filtered using Whatman No. 1 filter paper. The reddish brown filtrate was made up to 100 ml with Tyrode Ringer solution and stored in a refrigerator (t ≤ 4°C). Subsequently, any known aliquot from the concentrated extract represented a known weight of the pepper extract.

Determination of metabolic rate

About sixteen of the albino rats supplied were randomly sampled and placed into four sets (or batches) each containing four rats. These animals were placed in different cages at ambient laboratory conditions (temperature: 27 ± 2°C; relative humidity 79 ± 2%). All the animals were fed on the usual rat marsh (obtained from the Animal House, University of Lagos), with adequate water supplies. In addition to their normal diet, each set containing four rats respectively was also fed on either of the pepper extracts; the first batch of four rats were placed on *C. frutescence* extract, the second batch on *C. annum* extract, and the third batch were fed only the normal rat marsh and served as control. For each of the extracts, the calculated doses for animals of different body weights were diluted to the following range of concentrations: 3.0, 6.0, 9.0, 12.0 and 15.0 mg/ml.

After recording their initial body weight, rats of the extract treated group were fed orally by tube feeding same concentration of extract once a week for five weeks. Each week, the test organisms were fed a higher dosage of extract. After about 15 min following the administration of the calculated dose of extract, the rate of oxygen consumption was determined and the metabolic rate computed (Eckert and Randall, 1983). In addition, the metabolic rate of each of the rat in the batch was always determined a few minutes prior to the application of the extracts, at any given time the exercise was carried out. The metabolic rate was computed using indirect calorimetry (Eckert and Randall, 1983) from oxygen consumption and heat production was calculated as calories per square metre per hour as described by Chukwu et al. (2006).

Gross and histopathological effects of pepper extract

The cumulative effect of capsaicin application on the albino rats was investigated using the remaining batch of animals. The rats were randomly sampled and placed into three different groups, each containing five rats. They were placed into different cages. The animals were fed normally with adequate water supply. In addition, the first set of five were placed on *C. annum* extract, which were quickly prepared on each occasion when the animals were to be fed. The second batch of rats served as control, and these were given Tyrode mammalian ringer solution each time the first batch were fed with extracts.

The pepper extract was given to the animals orally by tube feeding every two days for a period of forty two days. On each occasion, the same dosage of extract, 300 mg/100 ml was always applied. After every seven days (one week), the weight of both experimental and control animals were taken. On the forty second day, two rats each from each of the batches was killed, and the gross pathological changes of the liver and gastro-intestinal tract were observed. The liver and gastrointestinal tracts were removed from both control and experimental groups. They were fixed in Bouin's fixative in order to prevent autolysis. The liver and gastrointestinal tracts were dehydrated using successive concentrations of ethanol, cleared in xylene, impregnated in paraffin wax, from which 10 µm longitudinal sections

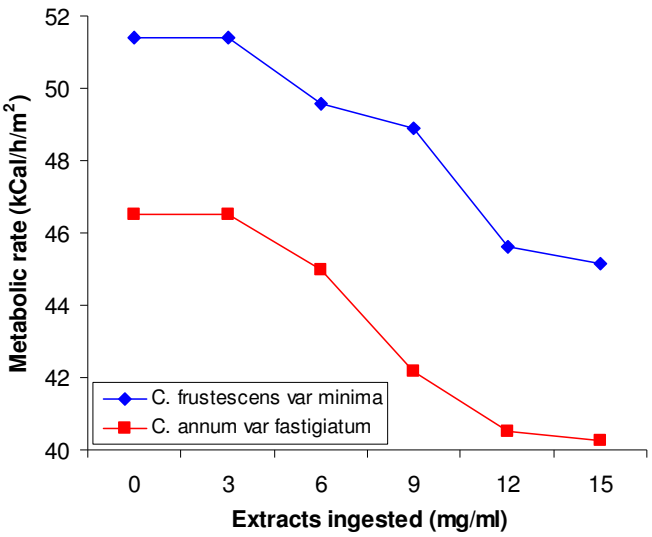

Figure 1. The effect of C. frustescens var minima and C. annum var fastigiatum extracts on the metabolic rate of male albino rats.

were cut for light microscopy. These were mounted on albumenized glass slides and stained with haematoxylin and eosin stain techniques. Thereafter, the sections were washed in distilled water, passed through ethanol again, mounted in Canada Balsam for examination and photomicrographs.

RESULTS

Effect of pepper extract on metabolic rate

The various species of pepper extracts had a rather negative effect on metabolic rate, bringing about a decrease with increase in concentration of extract. The *C. frutescence* extract produced a consequent decrease in rate of metabolism as the concentrations of the extract were increased (Figure 1). Similarly, *C. annum* extracts produced no observable change in metabolic rate at low concentration of 2 mg/ml of extract. At highest concentration of 15.0 mg/ml of extract, the magnitude of the change in metabolic rate was very high, 113.42% as compared to 112.14% for *C. frutescens* (Figure 1).

Effect of pepper extracts on the histophysiology of albino rats gross morphology

The animal fed on *C. annum var fastigiatum* were observed to have recorded significant (P>0.05) considerable weight losses over a period of 42 days with respect to control. The weight of the control animals did not vary much during the period, though very slight gain in weight was recorded on a few occasions (Figure 2).

The colour of the liver of the experimental animals fed on pepper extracts were observed to have a darker reddish colour when compared with control. However, no observable change in size of the liver was recorded in both experimental and control groups.

The structure of the liver in the control group showed hepatocytes displaying homogenous cytoplasm which were arranged in one or two cells thick muralia separated by sinusoids in a tubulo-sinusoidal pattern. In liver cells from experimental group, cytoplasm degeneration and loss of parenchymatous cell architecture were observed. Sinuses were packed with degenerate red blood cells, although relatively large isolated groups of macrophages were also scattered between the hepatic cords. In addition to the sites of pigment storage, every liver cell was crowded with numerous brown retractile granule which appear similar to the granular content of the macrophages, but there was an almost complete absence of granular debris in the stellate cells of Kupffer.

Structural changes were principally observed in the intestinal tract of animals treated with pepper extracts. Damage to intestinal mucosa appeared in most cells as indicated by slightly necrotic columnar epithelium and the appearance of sloughed cells in the intestinal lumen. Epithelial cell cytoplasm tended to be acidophilic. Striated borders of some areas were not well defined. Large numbers of cells at the tips of the villi had irregular nuclei. In the lamina propria, capillary congestion and minimal lymphatic infiltration were apparent.

The entire gastric surface and the glands were lined by simple columnar epithelium. Cells of four major types occur; surface mucous cells, neck cocoons cells, acidophilic parietal or oxyntic cells and the basophilic chief peptic cells. Only slight histopathological changes were observed in the gastric mucosa of the experimental animals. The pepper extract treatment also led to desquamatic necrosis of epithelial lining with increased mucous material. Some of the chief and parietal cells showed an appearance of pale basophilic cytoplasm and vacuolization. No observable changes were however seen in the lamina propria and the gastric glands associated with it.

DISCUSSION

From the results of this study, it was found that the various species of pepper brought about little or no change in basal metabolic rate at low concentrations and a slight decrease at high concentration of extract. The observed decrease was of greater magnitude in *C. annum* than in *C. frutescens* with the percentage decrease in basal metabolic rate at the highest concentration for the former (113.42%) than in the latter (112.14%). *C. annum* has a higher capsaicin potency and elicited a greater decrease in metabolic rate, possibly due to the greater sensation produced. The active principle

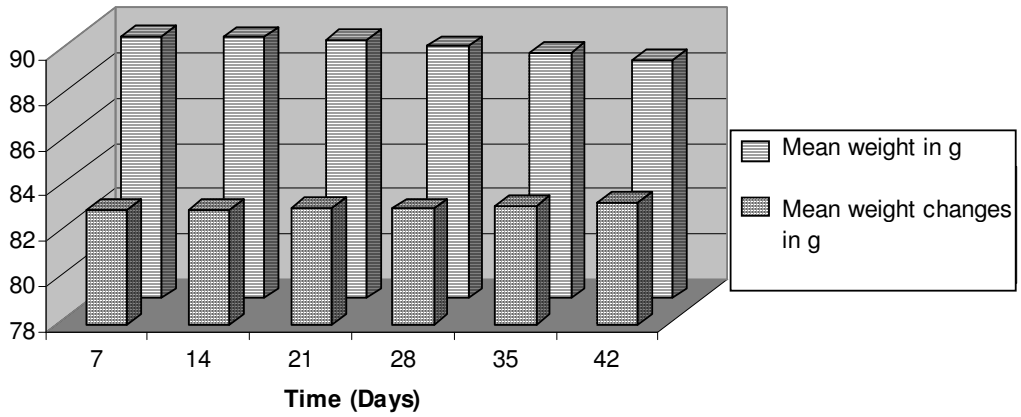

Figure 2. Weight changes in male albino rats following oral ingestion of same concentrations (300 mg/100 ml) of *Capsicum annum var fastigiatum* pepper extract over a period of 42-days.

responsible for this response pattern has not been identified but there is a possibility that capsaicin the active ingredient of pepper could be implicated. There is the possibility that the oral administration of the pepper extract could be acting in a similar manner as capsaicin which when administered peripherally to albino rats produced a hypothermic effect which failed to produce detectable changes in the metabolic rate (Glinsukon et al., 1979). According to Porszazz et al. (1957), the effects of acute intravenous administration of capsaicin on anaesthesized cats and dogs demonstrated production of Bezold – Jarish reflex (hypotension, bradycardia and apnea) with apnea being followed by increase in respiratory rate.

From the results of this investigation, severe histopathological changes were observed in the liver and gastrointestinal tract. Under the light microscope, the hepatocytes of the liver showed cytoplasmic degeneration, with sinuses packed with pigments of erythrocyte origin as well as refractive granules similar to the granular contents of macrophages. This observation probably explains the blotchy discolouration (i.e. darker colour) of the liver.

The direct effects of capsaicin and other naturally consumed stimulants on the gastro-intestinal tract have received less attention than the pharmacology and physiological effect. The cytotoxic activity of capsaicin on rat intestinal absorptive cells has been documented by Nopanitaya and Nye (1974). These authors observed mitochondrial as well as other subcellular structural alterations in these cells following intradoudenal or intragastric administration. They also suggested that capsaicin can profoundly influence oxidative phosphrylation and other energy linkage functions of the rat lives due to the mitochondrial and subcellular structural alteration of the cell. Lee (1963) reported that chronic feeding of various levels of capsaicin to rabbits resulted in hepatic and renal necrosis. Nopanitaya resulted in hepatic and renal necrosis. Nopanitaya (1973) working on toxic effect of natural capsaicin on the duodenal absorptive cells reported that severity of morphological alteration of those cells increased with time.

Damage to the intestinal and gastric mucosa was observed as indicated by slightly necrotic columnar epithelium and the appearance of sloughed cells in the intestinal lumen. According to Monsereenusorn and Glinsukon (1978), capsaicin had inhibitory effects on glucose absorption and hamster jejunum. The inhibitory mechanism may involve changes in the metabolic activities within the intestinal mucosal cells, or impairment in the ATP synthetic ability of the mitochondrial (Chudapogse and Janthasooth, 1976). This probably accounts for the loss in weight recorded in the experimental animals during the period of study.

The study illustrates that hot red pepper had direct toxic effect on absorptive cells, which were demonstrated as cellular damage by light microscope. The physiological consequence(s) of this ultra structural alteration should prove to be an area of fruitful investigations. This result proves that excessive consumption of pepper might result in chronic ulceration of the gastrointestinal tract. Nonetheless, the pain and pungent sensation of capsaicin in the fruit will control the consumption of the spice. Hence, the acute toxicity of capsaicin as a food borne substance in man would rarely occur.

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