academicJournals

Vol. 7(44), pp. 3247-3253, 25 November, 2013
DOI: 10.5897/JMPR11.406
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Effect of *Piper nigrum* on salivary gland of Wistar rat

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Accepted 28 April, 2011

Crushed *Piper nigrum* fruits are used for seasoning and flavouring of food. Some investigators have reported on its efficacy as an aid to the contraction of the uterus in the diet of post-partum women. This study aims at investigating the effect of *P. nigrum* fruits on the salivary glands for 3, 5 and 7 days of administration orally. Adult male and female Wistar rats (n = 30) weighing between 150 and 250 g were randomly assigned into experimental (n = 24) and control (n = 6) groups. The rats in the experimental groups received a mixed diet of feed consistency of crushed *P. nigrum* fruits to rat mash in a ratio of 50:50 (Group A, higher doses) and 25:75 (Group B, minimum doses) and water *ad libitum* for 3, 5 and 7 days. The rats in the control group received equal amount of rat mash without crushed *P. nigrum* fruits, for the same number of days. The rats were sacrificed on the 4th, 6th and 8th days. The stomach, small intestine and salivary glands were carefully dissected out and quickly fixed in 10% formol saline for histological and histochemical procedure. The body weights of the rats were recorded before and during treatment. The histological finding after 3 and 5 days administration of crushed *P. nigrum* fruits showed no significant changes in the histology of the salivary glands. There were vacuolations in the mucosa of the stomach and Brunner's glands of the duodenum of the experimental animals after 7 days administration of crushed *P. nigrum* fruits. The histological changes in the salivary glands were marked both in the experimental groups that received the higher doses (of feed consistency in the ratio of 50:50 of crushed *P. nigrum* fruits to rat mash) for 7 days. There was a slight decrease in the body weight in the experimental groups. This was not significant (P > 0.05). The results show that consumption of crushed *P. nigrum* fruits for 3 to 5 days may not be harmful, but consumption of it continuously for 7 days even when used with minimum doses may be detrimental to health.

Key words: *Piper nigrum*, salivary glands, detrimental, Wistar rats.

INTRODUCTION

Spices form the integral part of various cuisines all over the world. They are products of plants, which are mostly used for seasoning, flavouring and thus, enhancing the palatability and taste of foods, beverages and drugs (Parry, 1999; Manandhar, 1995). Plants used as spices are usually aromatic and pungent (Achinewu et al., 1995). Iwu (1993) had reported that these plants owe these properties to the presence of varying types of certain spices to these essential oils. It is indicated that essential oil. Macmillan (1984) the rich presence of essential oil and oleoresins associated the antiseptic and preservative property of determine the aromatic, flavouring and colouring properties in spices and condiments, as described by Dziezak (1989).

Crushed *Piper nigrum* fruits serve as a spicy flavouring in most African traditional culinary. It is consumed together with other ingredients to enhance palatability. Its aroma stimulates appetite by causing salivation as reported by Platel and Srinivas (2000).

In the Niger Delta area of Nigeria, these spices are

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collected from the Wildlife reserves. This spice and its herbs are used generally to prepare “pepper soups” which may be taken hot or cold and eaten especially during the cold, raining seasons. Achinewu et al. (1995) reported that this spice is particularly very important in the diets of post-partum women as an aid to the contraction of the uterus as it regresses to about its normal size.

The National Food and Drug Administration and Control (NAFDAC) directorate has included *P. nigrum* in the list of substances that are generally recognized as safe (GRAS) for their intended uses (Atal et al., 1985).

P. nigrum fruits (Black pepper) are produced by a woody, broad-leaved evergreen vine that is cultivated in the ecosystem of the Nigerian vegetation. It belongs to the family of Piperaeae. It is a tropical perennial climbing vine which grows on trees, poles or other forms of support to about 20 ft high. It has a life span of about 20 years (Gill, 1980). The leaves of the *P. nigrum* vine produce a mild pungent smell. This property enables it to be used in flavouring of food.

The fruits are generally harvested while they are still green in colour. The signal for harvesting is the reddening of the lowest fruits on the spike. The fruits are dried in the air in the shade. During this process, an enzyme contained within the pericarp (outer skin) turns the green fruit black, creating a substance known as piperine. These fruits are dried until the flesh around the single hard seed is wrinkled and grayish black. The piperine in the seeds provides the characteristics aroma and pungent flavour. The dried fruits are afterwards grounded or packed and sold as whole pepper corns.

P. nigrum fruits stimulate the appetite by the aroma. It is a spice used as a condiment to improve the flavour of foods, preservation of meals and as an essential ingredient in pepper soup. It is used as an aid in the relief of nausea and a carminative which reduces stomach and intestinal gas.

The Nigerian tribes have different names for this spice. The “Ibos refer to it as “Ozizi” while the “Efiks” and the “Ibibios” commonly refer to it as “Etikene and “Odusa” respectively. (Iwu, 1993). Whereas much work has been done on this fruit, no documented literature is on record as to its effect on the salivary glands. The objective of this study is to determine the effect of *P. nigrum* on the histology of the salivary gland, the relative safety in the consumption of *P. nigrum* and to ascertain if *P. nigrum* could adversely affect salivary gland particularly as it is used locally as an aid to relieve digestive disorders.

MATERIALS AND METHODS

Equipment/ Instruments

The materials used included the following: forceps, surgical blade, scapel knife, 10% formal saline, specimen bottles, dissecting kit, dissecting board, cotton wool, toilet roll, weighing balance (mettler Toledo weighing balance), disposable gloves, rotary microtome, water bath, oven, xylene, 70% alcohol, 90% alcohol, absolute alcohol, haematoxylin, eosin, egg albumin, distilled water, paraffin wax, wooden blocks, mountant (Distrene plasticizer and xylene), slides and cover slips.

Care and management of animals

Thirty-five (35) adult Wistar rats of both sexes weighing between 150 and 250 g (aged 12 weeks) were used in this study. They were procured from the animal house of the Department of Anatomy and Department of Zoology, University of Benin, Nigeria. The rats were maintained in the Animal Holdings of the Department of Anatomy, University of Benin, Benin City, Nigeria.

They were fed with rat mash [which contained crude protein (14.5%), crude fat (4.8%), crude fiber (7.2%), crude ash (8%), calcium (0.8%), phosphorous (0.25%), lysine (0.6%), methionine (0.29%), methionine + cystine (0.52%), vitamin A (8,000 I.U.), vitamin C (50 mg), manganese (30 mg), zinc (30 mg) and sodium (0.155%)]. The feed was manufactured by Bendel Feed and Flour Mills Limited Ewu, Edo State, Nigeria. The feed was purchased from local feed shop in Benin City. Water was given *ad libitum.* All the animals were carefully assessed, and confirmed to be free of any pathological conditions following the period of acclimatization to the experimental conditions, which lasted for 2 weeks. During the period of acclimatization, the body weights of the rats were recorded. The animals were kept in the animal room with controlled environmental temperature and with a cycle of 12 h of day light and 12 h of darkness.

Preparation of fruit (*P. nigrum*) powder

The fresh fruits of *P. nigrum* were purchased in large quantities from Oba Market in Benin City between March and May, 2006 and were identified and authenticated at the Department of Botany, University of Benin, Nigeria.

The fruits were kept in an aerated shade to dry in order to prevent the loss of volatile enzymes for 7 days. The dried fruits were ground into a powdery form and weighed. This process was carried out at the Laboratory in the Department of Pharmacognosy, University of Benin City, Nigeria.

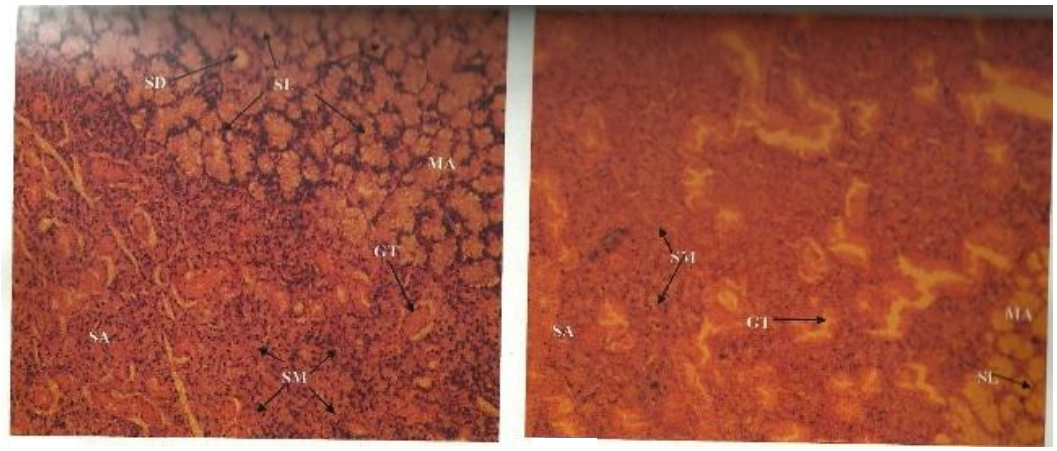
Experimental design

The Wistar rats were randomly divided into 3 groups (2 experimental and 1 control). The experimental groups were labeled A and B. respectively. Groups A and B comprised of 15 rats each. The control group comprised of 5 normal Wistar rats.

Three classes of food mixtures (mixed diet) were designed:

(1) Animals in the experimental Group A were fed with a mixed diet of *P. nigrum* mixed with rat mash having a consistent weight ratio of 25:75 for *P. nigrum* and mash.
(2) Group B animals were placed on a diet of 50:50 ratio by weight of *P. nigrum* to rat mash mixed diet formation.
(3) The control group received equal amounts of rat mash alone without *P. nigrum*

The *P. nigrum* diet was thoroughly mixed with mash in the mixed diet in a specially prepared container fixed to the floor of the cage to avoid spillage of the feeds during feedings by rats. The feeds and the animals were weighed daily at a fixed time of 9 am. The feed was changed daily and the quantity of food consumed each day recorded. The experimental animals were kept on the diet regimen for 3, 5 and 7 days. Both the experimental and control groups of animals were sacrificed by stunning followed by cervical dislocation method on the 4^{th}, 6^{th} and 8^{th} days.



A B

Figure 1. Micrographs of H&E stained sections of the salivary glands from Wistar rat. A, Control; B, experimental animal.
The experimental animal was placed in a food regimen composed of 25:75 crushed *P. nigrum* fruits to rat mash for 3 days. Both the submandibular and sublingual glands of the experimental animal appeared normal.
GT, Granular tubule; H&E, haematoxylin and Eosin; SL, sublingual gland; SM, submandibular gland; SA, serous acinus; SD, striated duct; MA, mucus acinus (×400).

Dissection of the salivary gland

The abdomen and neck region were incised by a median longitudinal incision through the skin to expose the salivary glands under the lower jaw. The anterior abdominal wall muscle was incised to expose the gut in the abdomen. The salivary gland was carefully dissected out using a sharp scapel knife. The tissues were removed and were fixed in 10% formal saline in specimen bottles for routine histological studies using Haematoxylin and eosin (H&E) staining techniques (Drury et al., 1976).

Tissue processing

Histological techniques

Following fixation, the tissues were further processed by dehydration through ascending grades of alcohol. The first grade of alcohol used was 70% for a day, followed by 90% overnight and finally two changes of absolute alcohol the following day. After dehydration, the tissues were treatment with xylol (70% xylene / 30% absolute alcohol) and then with xylene for a day followed by infiltration in three changes of paraffin at 60°C for 2 days, using an oven. Lastly, the tissues were transferred into an embedding medium (fresh paraffin wax,) followed by blocking. Sections of about 5 microns thick were cut using a rotary microtome.

H&E staining method

Sections were de-waxed for 2 min in each of two changes of xylene and were transferred into absolute alcohol for removal of xylene for 1 min and were stained with iron haematoxylin for 20 min. They were washed in running tap water for 2 to 3 min. Sections were differentiated in 1% acid alcohol for a few seconds. Blued in running tap water for 5 min, and counter-stained with 1% eosin for 3 min, then rinsed in water. They were dehydrated through ascending grades of alcohol (70, 90 and 100%) for 1 min each, cleared in xylene for 1 min and mounted in distrene, plasticizer and xylene (DPX) (Drury and Wallington, 1967).

The body weights of the rats were measured daily using the weighing balance.

Statistical analysis

The values of all the morphometric analysis were compared statistically using SPSS Package

Records of the histological and histochemical results were obtained by photomicrography using a microscope with a camera at the Department of Anatomy, University of Benin, Benin City as shown in Figures 1 to 6.

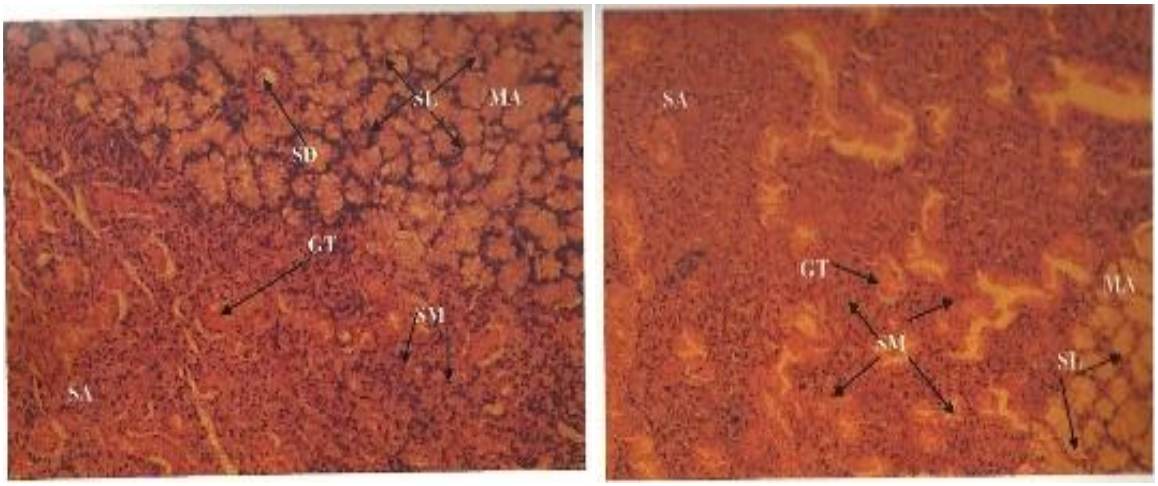
RESULTS

Control group

H&E staining of the salivary glands showed (a) sub-mandibular gland and (b) sublingual gland. The staining reaction of the submandibular gland and the basal cells of the sublingual gland were basophilic. The submandibular gland is composed of both serous and mucous secreting acinar cells, but the serous acinar cells predominate while the sublingual gland is composed predominantly of mucous secreting acinar cells with serous demilumes. The ducts in the submandibular gland contain acidophilic storage granules. The sublingual gland lies superior to the submandibular gland separated from it by loose connective tissues septa. The mucous acinar cells in both the submandibular and sublingual glands are not stained by H&E.

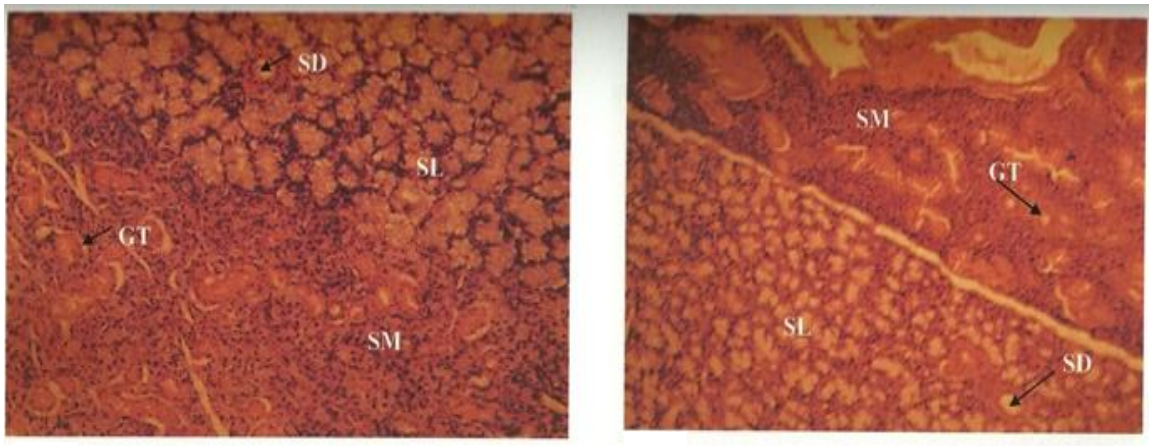
Experimental group

The histological sections of the salivary glands of the



A B

Figure 2. Micrographs of H&E stained sections of the salivary glands from Wistar rat; A, Control; B, experimental animal.
The experimental animal was placed in a food regimen of mixed diet composed of 25:75 crushed *P. nigrum* fruits to rat mash for 5 days. There were no observable histological changes in the glands.
GT, Granular tubule; H&E, haematoxylin and Eosin; SD, striated duct; SA, serous acinus; MA, mucus acinus; SL, sublingual gland; SM, submandibular gland (Mag. about ×400).



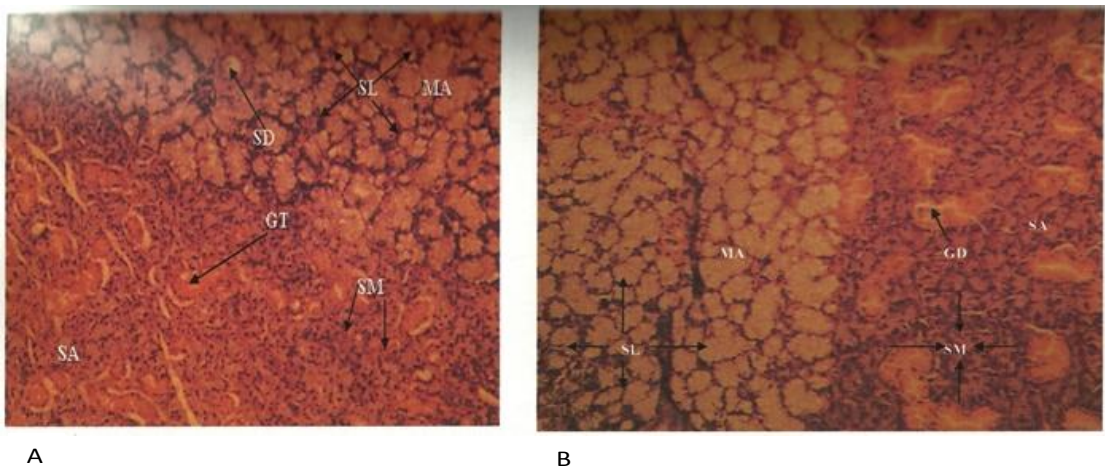
A B

Figure 3. Micrographs of H&E stained sections of the salivary glands showing the sublingual and submandibular glands from Wistar rat; A, Control; B, experimental animal.
The experimental animal was placed in a food regimen of mixed diet composed of 25:75 crushed *P. nigrum* fruits to rat mash for 7 days. The sublingual salivary gland of the experimental animal appeared shrunken while the striated duct remained normal. In the submandibular gland, the acini appeared shrunken. The submandibular gland with its granular tubules appeared normal.
SL, Sublingual gland; SM, submandibular gland; GT, granular tubule; SD, striated duct; H&E, Haematoxylin and Eosin (Mag. about ×400).

experimental animals placed on food regimen composed of crushed *P. nigrum* fruits mixed with rat mash in the ratio of 25:75 and 50:50 for 3 and 5 days showed no degenerative lesions. However, consumption of crushed *P. nigrum* fruits for 7 days by the experimental animals in a ratio of 25:75 and 50:50 of crushed *P. nigrum* fruits to rat mash, showed a shrunken appearance of the salivary glands with a reduction in the size of the mucous acinar cells.

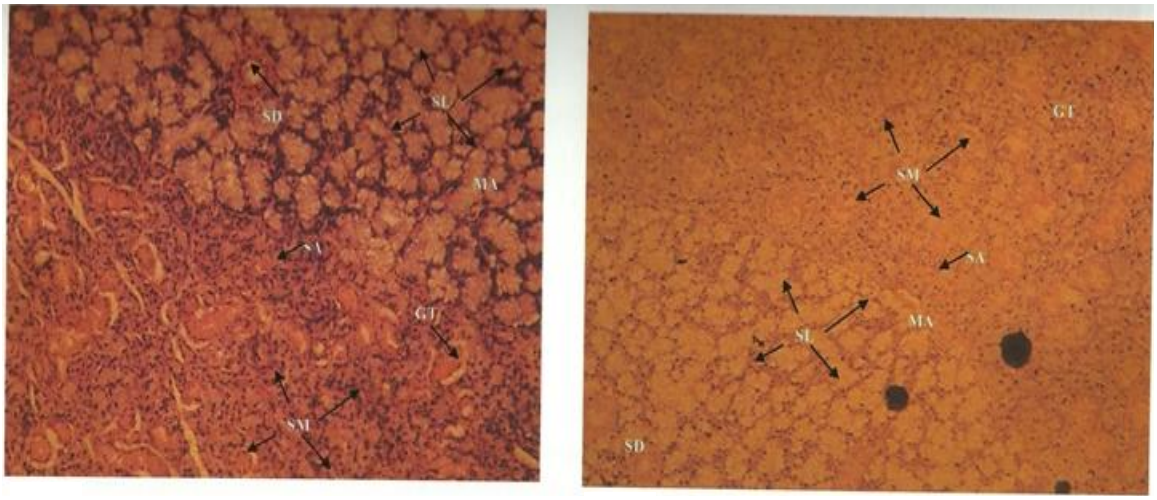
DISCUSSION

In this study, the effects of crushed *P. nigrum* fruits mixed in ratio of 25:75 and 50:50 with rat mash fed to rats of the Wistar strain for 3, 5 and 7 days were determined. However, prolong feeding of mixed diet in a ratio of 25:75 for 5 days produced debilitating effects which showed as degenerative changes. The mucous acini of the sublingual



A B

Figure 4. Micrographs of the salivary glands showing the H&E stained sections made up of submandibular and sublingual glands from Wistar rat; A, Control; B, experimental animal.
The experimental animal was placed on 3 days regimental feeding and a mixed diet composed of 50:50 crushed *P. nigrum* fruits to rat mash. There were no observable histological changes in the glands.
GT, Granular tubule; MA, mucus acinus; SA, serous acinus; SL, sublingual gland; SM, submandibular gland; H&E, haematoxylin and Eosin; SD, striated duct (Mag. about ×400).

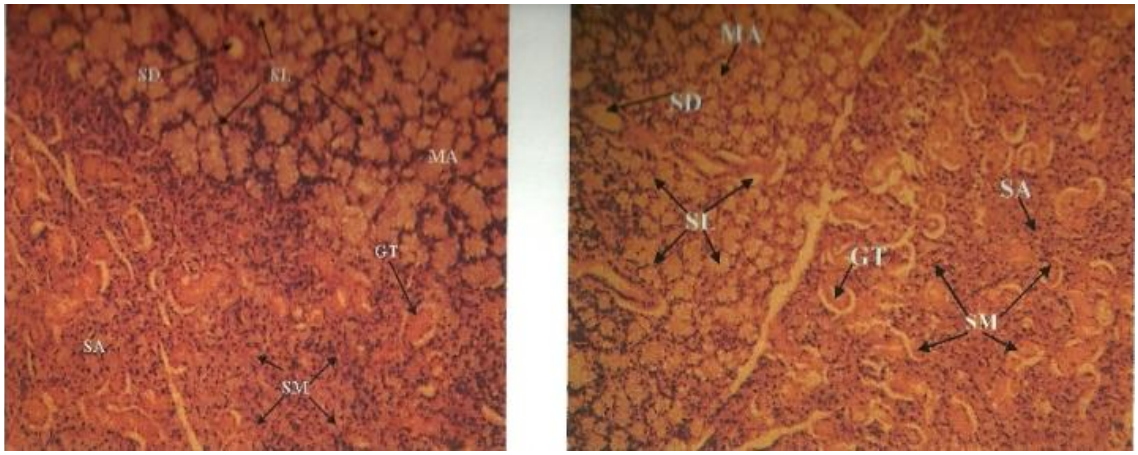


A B

Figure 5. Micrographs of the salivary glands of Wistar rats showing H&E stained sections from: A, Control; B, experimental animal.
The experimental animal was placed on a food regimen of mixed diet composed of 50:50 crushed *P. nigrum* fruits to rat mash for 5 days. There were no observable histological changes in the glands.
GT, Granular tubule; MA, mucus acinus; SA, serous acinus; SL, sublingual gland; SM, submandibular gland; SD, striated duct; H&E, haematoxylin and Eosin; (Mag. about ×400).

sublingual glands appeared shrunken but the structure of the submandibular glands appeared to remain normal. In the salivary glands, acini of the sublingual glands appeared shrunken while the striated ducts appeared normal. In the case of the submandibular, tubules appear bunched together.

The lack of observation changes in organs of experimental animals after 3 days; regimen probably enhances and encourages the local use of crushed *P. nigrum* fruits in the treatment of digestive disorders, as alleged by its consumers and reported by Ndukwu and Ben-Nwadibia, (2003). The non-toxicity of crushed *P. nigrum* fruits in the stated ratio after 3 days consumption by the experimental rats could be explained as a consequence of the transient time that the food remains in the stomach and possible the actions of the digestive enzymes secreted in the stomach. This buttresses the work carried out by Platel and Srinivas (2000) that showed that when added



A B

Figure 6. Micrographs of the H&E stained sections of the salivary glands from Wistar rats showing; A, Control; B, experimental animal.
The experimental animal was placed on a food regimen of mixed diet composed of 50:50 crushed *P. nigrum* fruits to rat mash for 7 days. The glands from the experimental animals appeared shrunken. The mucus acinar appeared reduced in size while the striated duct appeared normal. The submandibular acini also appeared reduced in size and the granular tubules shrunken such that they appeared bunched together in the submandibular gland.
GT, Granular tubule; MA, mucus acinus; SA, serous acinus; SL, sublingual gland; SM, submandibular gland; SD, striated duct; H&E, haematoxylin and Eosin; (Mag. about ×400).

to food, crushed *P. nigrum* fruits stimulates the appetite by the secretion of digestive enzymes such as pancreatic amylase, trysin, chymotrypsin and lipase. Crushed *P. nigrum* fruits which are known for its stimulating effects on digestive enzymes could have been expected to have degenerative effects on the surface membranes of the digestive system, but this not observed after the 3 days of the feeding regimen. Crushed *P. nigrum* is readily absorbed from the gastrointestinal tract. It enhances the bioavailability of a broad range of nutrients including those that are water soluble, fat soluble, amino acid molecules and herbs as reported by Johri and Zutshi (1992b). *P. nigrum* also promotes the bioavailability of various structurally and therapeutically diverse drugs. A concise mechanism of its bioavailability enhancing action is not well understood. Khajuria and Kivcak (1998) in his studies recorded that *P. nigrum* is absorbed very fast across the intestinal barrier forming non-polar complexes with drugs and solutes thus, increasing permeability across the barrier. This factor may probably constitute the reason why the food of regimen mixed diet composed of 25:75 and 50:50 of crushed *P. nigrum* fruits to rat mash fed to the experimental rats for 3 days rendered no histological effect to the salivary glands. *P. nigrum* also enhances the absorption of nutrients. It promotes rapid absorption of nutrient from the gastro intestinal tract because it is lipophilic (has an affinity for fatty tissue) (Khajuria and Kivcak, 1998). *P. nigrum* may interact with the lipid component of the intestinal cell membrane to facilitate enhanced nutrient permeability and also stimulates glutamyl transpeptidase, an enzyme that promotes amino acid uptake from the gastro intestinal tract as supported by Johri and Zutshi (1992a).

In the salivary glands, the serous secreting acinar cells of the submandibular glands showed no distortions in the sections of the experimental animals fed with a food regimen composed of 25:75 and 50:50 of crushed *P. nigrum* fruits to rat mash for 3 and 5 days. The mucous secreting acinar cells of the sublingual glands bound by demilunes were clearly defined. The aroma and soothing sensation of crushed *P. nigrum* fruits in the mouth causes salivation and ptyalin which is the digestive enzyme in saliva acts readily upon the food. In the sections of the salivary glands, the result of normal secretion of enzymes as shown by experimental animals placed on food regimen composed of crushed *P. nigrum* powder mixed with normal rat mash in a ratio of 25:75 and 50:50, respectively for 3 days showed no degenerative lesions. This supports the work of Platel and Srinivas (2000) who found that *P. nigrum* enhanced digestive enzyme secretion and ultimately improved thermogenesis (Bhat and Bennett, 1999). This is the reason for persons who consumed normal meals fortified with *P. nigrum* palatability never suffered but enjoyed the meal which enhanced generation of heat because of the presence of the dried *P. nigrum* fruits.

There was a decrease in the body weight of the experimental group weight of the animals. This could be explained as a result of thermogenesis enhanced by the secretion of the digestive enzymes. Thermogenesis is a metabolic process which generates energy at the cellular level in the body by utilizing the nutrient consumed. This

process helps to burn up excess fats in the tissues as reported by Vijakumar et al. (2004). The active principle in crushed *P. nigrum* fruits which exhibits this function is peperine. It has the potency to reduce high fat diet oxidative stress to cells.

P. nigrum enhances thermogenesis, the production of heat in the body. In the process of digesting food, energy is used. This is in the production of heat and the feeling of warmth that often accompanies a meal. It stimulates the release of catecholamines that initiates the process of thermogenesis in the gastro intestinal tract (Kawado et al., 1988), thereby increasing the energy available for digestion. *P. nigrum* enhances and encourages thermogensis of lipids (fat molecules) and accelerates energy metabolism in the body. It traps and enhances the thermogenesis of saturated fat which are most difficult to be used by physical activity. Although thermogenesis has been identified as a key factor in maintaining weight loss, it also plays an integral absorption by increasing the thermal energy sufficient to 'power up' the mechanism of thermogenesis (Bhat et al., 1999). This results in increased metabolic processes that provide a more efficient mode of nutrients (that is, vitamins, minerals, amino acids etc) transportation into the blood.

The increased rate of metabolism and the active state of activities displayed by the experimental animals may probably have been as a result of the stimulation of the endocrine system. *P. nigrum* is supportive to the digestive glands. It also increases cellular oxygenation. This confirms the work carried out by Reanmongkol and Raman, (1988). *P. nigrum* stimulates the taste buds that signals and alerts the stomach, thereby increasing the secretion

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