The Effect of Capsaicin and Eugenol on the Submandibular Salivary Gland of Albino Rats

Thesis

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INTRODUCTION & REVIEW

OF LITERATURE

Natural flavors are widely used in various foods, cosmetic and pharmaceutical products. These kinds of additives are applied as colors, preservatives, aroma and tasting agents. The large-scale use of certain food flavors requires accumulation of toxicological data on these substances **(Shoeibi et al., 2009)**.

Capsaicin

Capsaicin is a pungent principle of hot red pepper. It is used in spices, food additives and drugs. **(Suzuki and Iwai, 1984)**

Red pepper (Capsicum frutescens L.) is widely used as a spice for flavoring foods, particularly in South- East Asian and Latin-American countries. The major active ingredients of red pepper are pungent capsaicinoids (capsaicin and dihydrocapsaincin), antioxidant vitamins (ascorbic acid and vitamin E), carotenoids (β-carotene and β-cryptoxanthine) as well as several organic acids and minerals **(Antonious et al., 2006; Conforti et al., 2007).**

Systemic effects of capsaicin:

Jonietiz (1982) studied the effect of capsaicin and red pepper on activities of disaccharidase such as maltase, lactase, and sucrase in the rat jejunum. The researchers found that jejunal disaccharidase activities were inhibited in vitro by capsaicin in a concentration range of 10^{-7} to 10^{-2} mole after 1 hour incubation. On the other hand jejunal disaccharidase activities of rats fed red pepper and capsaicin did not show significant differences compared with those of control rats in vivo.

Kawada et al. (1986) studied capsaicin effect on the level of serum triglycerides in an experiment using male rats fed a diet containing 30 % lard. Capsaicin was supplemented at 0.014 % of the diet. The level of serum triglycerides was lowered when capsaicin was present in the diet than when it was not. These results indicated that capsaicin stimulated lipid mobilization from adipose tissue and lowered the perirenal adipose tissue weight and serum triglycerides concentration in lard-fed rats.

Capsaicin is used as a topical analgesic against arthritis pain and inflammation **(Deal et al., 1991).** It binds to the same group of nociceptors which leads to the sensation of pain from heat and acid and reduces pain and

inflammation by depleting the neurotransmitters signaling pain **(Szallasi and Blumberg, 1999; Caterina and Julius, 2001 and Julius and Basbaum, 2001)**.

Mozsik et al. (2005) reported that Capsaicin in low concentration range (1-8 µg/mL, 100 mL) given by nasogastric tube before gastric injuries induced by ethanol or indomethacin protected the stomach. This was attributed to stimulation of the sensory nerve endings by capsaicin.

The beneficial influence of the known hypocholesterolemic spice principle capsaicin on the susceptibility of low-density lipoprotein to oxidation in normal and hypercholesterolemic condition was studied by **Kempaiah et al. (2005)**. The authors reported that in rats rendered hypercholesterolemic by maintaining them on a cholesterol enriched diet for eight weeks, inclusion of capsaicin (0.015%) in the diet produced significant hypocholesterolemic effect. Hepatic lipid peroxidation was significantly decreased by dietary capsaicin in normal rats.

It is thought that when red pepper is consumed in excessive amounts, it leads to "gastric ulcers" in view of its irritant and likely acid secreting nature **(Satyanarayana, 2006)**. Persons with ulcers are advised either to limit or avoid its use. However, investigations carried out in recent

years have revealed that red pepper or its active principle "capsaicin" is not the cause for ulcer formation but a co-factor. Capsaicin does not stimulate but inhibits acid secretion. It stimulates alkali, mucus secretions and particularly gastric mucosal blood flow which help in prevention and healing of ulcers. Capsaicin acts by stimulating afferent neurons in the stomach and signals for protection against injury causing agents **(Satyanarayana, 2006)**.

Effects of capsaicin on salivary glands:

The effects of dietary capsaicin were investigated by **Katsukawa and Ninomiya (1999)** on the rat submandibular gland and its secretions. Several groups of animals were offered either control diet or diet containing capsaicin (from 0.0001 to 0.1%) for seven days. The relative weight of the salivary glands in capsaicin diet groups increased in a dose dependent fashion, and cystatin S substances appeared in the submandibular saliva. It was suggested that dietary capsaicin induced cystatin S substances in submandibular saliva by stimulating the reflex arc involving the glossopharyngeal nerve. These proteins likely facilitated ingestion of diets containing the irritating substance.

Katsukawa *et al.*, (2002) reported that dietary capsaicin consumed by rats over several days induced cystatin S substances in submandibular saliva. The results suggested that cystatins were included in the salivary proteins induced by capsaicin and that they contributed to enhanced ingestion of the capsaicin diet. The authors added that the induction of salivary cystatins may be triggered by irritation of the oral mucosa by capsaicin.

Eugenol

Eugenol exhibits pharmacological effects on almost all systems. It possessed significant antioxidant, anti-inflammatory and cardiovascular properties. The compound is a very promising candidate for useful applications, and the design of new drugs based on the pharmacological effects of eugenol **(Pramod et al., 2010)**.

Systemic effects of eugenol:

Pulla Reddy and Lokesh (1996) reported that the intraperitoneal injection of iron showed hepatic damage in male wister rats. This damage was measured by an increase in lipid peroxides which correlated with elevated liver serum enzymes. Oral administration of spice principles, including

eugenol (100 mg\kg body weight), for ten days was associated with lowered liver and serum lipid peroxide levels.

The inhibitory actions of eugenol on intracellular calcium concentration and the contractions induced by excess extracellular potassium concentration in rabbit thoracic aorta were investigated by **Nishijima et al. (1999)**. Application of excess potassium solution produced contraction of thoracic aorta and increased the intensity of the calcium fluorescence signal. Pretreatment with eugenol in different doses reduced both the amplitude of contraction of thoracic aorta and the intensity of the calcium fluorescence signal. It was concluded that eugenol relaxed the rabbit thoracic aorta while suppressing the calcium sensitivity and both the uptake and extrusion mechanisms for calcium. Moreover, eugenol may produce its actions partly through metabolic inhibition.

Eugenol has biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties **(Lee & Shibamoto, 2001)**. It is used traditionally as flavoring agent and antimicrobial material in food **(Huang et al., 2002 and Velluti et al., 2003).**

Tiku et al. (2004) studied the radioprotective properties of eugenol. Different doses of eugenol (75, 150 and 300 mg/kg) were administered to Swiss Albino mice before exposure to 1.5 Gray of gamma radiation. Eugenol was also tested against different intensity (0.5, 1, 1.5 and 2 Gray) of radiation. It was found to afford significant radioprotection in the form of significant reduction in the frequencies of micronucleated polychromatic erythrocytes with the three doses of eugenol used. The researchers concluded that eugenol exerted it radioprotection through its antioxidative properties.

AI-Attar and Zari (2007) suggested that ginger oil, clove oil and their combination supplementation may act as antioxidant agents. These oils could be an excellent adjuvant support in the therapy of streptozotocin (STZ) induced diabetes mellitus and its complications in Wister rats. The authors found that treatment with eugenol decreased the values of blood glucose, triglyceraldhydes, cholesterol, *Low density lipoprotein* cholesterol, total protein, creatinine, urea, uric acid, alanine aminotransferase test and aspartate aminotransferase test in diabetic rats. They added that the eugenol acts like insulin in hepatocytes and hepatoma cells by reducing phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression. Additionally,

eugenol increased the activities of tissue antioxidant enzymes.

The chemoprotective effect of clove, chili and cardamom in correcting iron overload induced liver injury, oxidative stress and serum lipid profile in rat models was studied by **Sadeek and Abd El-Razek (2010).** The authors reported that all parameters of lipid profile and total bilirubin showed significant decrease by intraperitonial injection of clove, chili, and cardamom in male wister rats. On the other hand, the mean high density lipoprotein cholesterol and the activity of serum catalase were increased. The authors suggested that the chemoprotective effect of clove, chili, and cardamom was attributed to chelation with iron followed by excretion of the complex.

Oral and dental effects of eugenol:

Hume (1983) stated that eugenol is a natural pungent substance and the chief constituent of clove oil. It is commonly used in dental clinics for the sedation of toothache, pulpitis and dental hyperalgesia. It is included in various endodontic medications and applied directly to the teeth. It penetrates the dental pulp tissue and can enter the bloodstream.

The major constituent of betel quid (with or without tobacco) is eugenol. This betel quid is widely used in the India, Asia and the Pacific region**. Jeng et al., (1994)** studied the effects of eugenol from betel quid on oral mucosal fibroblasts in vitro at concentrations higher than 3mmol/L. Eugenol was cytotoxic to oral mucosal fibroblasts in a concentration and time-dependent manner. In addition, eugenol was found to decrease cellular ATP level and it inhibits lipid peroxidation in a concentration and time-dependent manner. The researchers concluded that frequent exposure of oral mucosa to a high concentration of eugenol (more than 3mmol/L) during the chewing of betel quid might be involved in the pathogenesis of oral submucous fibrosis and oral cancer via its cytotoxicity.

Eugenol was evaluated for its therapeutic efficacy in the treatment of experimental oral candidiasis induced by Candida albicans in immunosuppressed rats **(Chami et al., 2004)**. The anticandidal activity was analyzed by microbiological and histopathological techniques. The untreated control animals showed numerous hyphae on the epithelium of the dorsal surface of the tongue. When rats were treated with eugenol, only few focalized zones of the dorsal surface of the tongue were occupied by hyphae. The

authors concluded that eugenol could be used as therapeutic agent for oral candidiasis**.**

Jadhav et al., (2004) stated that eugenol is an integral part of the dentist's kit due to its analgesic, local anesthetic, anti-inflammatory, and antibacterial effects. It is used in the form of a paste or mixture as dental cement, filler, and restorative material. The authors conducted a study to evaluate controlled release mucoadhesive tablets incorporating 10 mg eugenol for gingival application used for the treatment of periodontal diseases; this mucoadhesive formulation provided controlled release for a period of eight hours, which was advantageous over conventional use. The investigators reported an increased potential of eugenol as an antibacterial and local analgesic substance.

Data on the effect of capsaicin and eugenol on the submandibular salivary gland was scarce. So, the present study aimed to investigate the effect of capsaicin, eugenol and their combination on the submandibular salivary gland of the albino rats histologically and ultrastructurally.

AIM OF THE STUDY

The aim of the present study was to investigate the effect of capsaicin, eugenol as well as their combination on the submandibular salivary gland of albino rats. The investigation was conducted using light and transmission electron microscopes.

MATERIALS AND METHODS

Thirty five adult male albino rats (weighing about 250 ± 20 gm. each) were used in this study. Animals were recorded in "The Medical Research Center", Faculty of Medicine, Ain Shams University. The animals were housed in wire mesh dated cages. They were fed certified pelleted diet and tap water ad libtium. Temperature and humidity conditions were controlled as possible on housing the animals during the experimental period.

The material (capsaicin and eugenol) used in this study were purchased from **Sigma chemical co., St. Louis, Mo, USA**. The olive oil was purchased from Egyptian market. The animals were divided into the following groups:

Group I: (Control group)

It consisted of 5 rats.

Group II: (Capsaicin group)

This group consisted of 10 rats subdivided into 2 subgroups (5 rats each).

Subgroup IIa:

The animals daily received 0.5 ml distilled water by oro-oesophageal tube after morning meal and was served as +ve control for subgroup IIb.

Subgroup IIb:

The animals daily received capsaicin dose was equivalent to 0.1 mg/ kg body weight (**Jonietz, 1982)** dissolved in 0.5 ml distilled water by oro-oesophageal tube after morning meal.

Group III: (Eugenol group)

This group consisted of 10 rats subdivided into 2 subgroups (5 rats each).

Subgroup IIIa:

The animals daily received 0.5 ml olive oil by oro-oesophageal tube after morning meal and served as + ve control for subgroup IIIb.

Subgroup IIIb:

The animals daily received eugenol dose was equivalent to 2.5 mg/kg body weight **(The Joint Food and Agricultural Organization (FAO)/World Health Organization (WHO)**