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PROTECTIVE AND THERAPEUTIC EFFECTS OF PROPOLIS AGAINST MONOSODIUM GLUTAMATE (MSG) TOXICITY IN RAT BRAIN: HISTOPATHOLOGICAL STUDY

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Abstract:-

Monosodium glutamate (MSG) is the salt of nonessential glutamic acid. It has a property to enhance the perception that flavors are well blended and full-bodied. Propolis is a resinous substance collected by honeybees from the bud and bark of certain trees and plants. It has been used in folk medicine from ancient times in many countries. The present study aimed to investigate the protective and curative effect of propolis against MSG on the rat brain. Accordingly, fifty male albino rats were divided into five groups. The first group served as control, whereas the second group was administered propolis at an oral daily dose of 200 mg/kg b. w. for eight weeks. The third group received MSG 1 g/kg b. w. for eight weeks. The fourth group (protective group) was initialy administered with propolis alone for 4 weeks, and followed by MSG in association with propolis for 4 weeks. The fifth group (therapeutic group) was first given MSG alone for 4 weeks and secondly administered propolis in association with MSG for 4 weeks. At the end of four and eight weeks, brain tissues were collected for histopathological study purposes. Histopathological studies of MSG-treated rats displayed deleterious alterations in brain tissues as indicated by perivascular oedema and cuffing. Some blood vessels were congested and there was vacuolation of some neurons. The protective group showed gliosis in the cerebrum. There were still some odematous changes and perivascular cuffing which were still persistent. Propolis extract in the curative group caused active hyperaemia of the brain, blood vessels were congested and showed perivascular oedema and some neurons exhibited a marked vacuolation. It may be concluded that the results confirm the toxic effect of MSG on the brain and the protective effect of propolis, especially when administrated as a propolis alone.

Keywords:-*Brain, Rats, Monosodium Glutamate, Propolis, Histopathology.*

INTRODUCTION

Glutamate is ubiquitous in nature and is present in all living organisms. It is the principal excitatory neurotrammitter in central nervous system. Trade names of glutamate include monosodmm glutamate (MSG), also known as sodium glutamate, umami, ajinamoto, vetsin and accent is a sodium salt of non-essential amino acid glutamic acid [1]. It is used as a food additive and is commonly marketed as a flavor enhancer [2]. Despite its taste stimulation and improved appetite enhancement, reports indicate that MSG is toxic to human and experimental animals [3, 4]. It has been reported that MSG has neurotoxic effects resulting in brain cell damage [5], retinal degeneration, endocrine disorder and some pathological conditions such as addiction, stroke, epilepsy, brain trauma, neuropathic pain, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis [6]. MSG administration exerted significant elevation of the mean body weight, absolute and relative kidney weights, serum urea, creatinine, sodium (Na^+), cholesterol, TG, HDL, LDL, VLDL and MDA activities and decrease in potassium (K^+), total protein and albumin [7]. Glutamate in high doses produced neuroendocrine abnormalities [8], neurodegeneration, neurotoxicity [9] and oxidative damage in different organs [10, 11]. In addition, investigators postulated that MSG induced alteration in mitochondria lipid peroxidation and antioxidant status in different brain regions as the cerebral hemisphere, cerebellum, brain stem and diencephalon [12, 13]. Propolis is a popular folk medicine possessing a broad spectrum of biological activities [14]. It has recently gained popularity as a healthy food in various parts of the world because it promotes health and prevents diseases [15]. Furthermore, propolis extract exhibits an inhibitory effect on the expression of inducible nitric oxide synthase and nitric oxide production [16], and also acts as antifungal [17], anti-hyperalgesic [18], antiparasitic [19], immunostimulatory [20], immunomodulatory [21] and neuroprotective [22]. Propolis extract showed significant improvement in the mean body weight, absolute and relative kidney weights, serum urea, creatinine, sodium (Na^+), cholesterol, TG, HDL, LDL, VLDL and MDA activities and decrease in potassium (K^+), total protein and albumin [7]. As rats were exposed to CCl_4 followed by treatment with propolis extract, light microscopical studies showed considerable protection in liver and kidney with propolis treatment [23]. The aim of this study is to investigate the protective and therapeutic effects of propolis against MSG toxicity on brain of albino rats.

MATERIALS AND METHODS

Fifty weanling male albino rats (*Rattus norvegicus*), each weighs between 75 and 95 g were used throughout the present study. The animals were housed in groups of five in standardized cages and were located in the same room with constant environmental conditions such as temperature (22±3°C) and humidity (50-60%). They were supplied with enough rat feed and drinking water *ad-libitum*. All animals were allowed to acclimatize in the running environment for one week before the commencement of the study which lasted for eight weeks. The chemicals used, monosodium glutamate (MSG) with 99% purity and propolis, were purchased from Sigma Chemical Company (USA).

Experimental Animal Grouping:

The animals were divided into 5 equal groups, each contains 10 male rats:

1) The Control Group: Animals of this group received distilled water daily by oral gavage for eight weeks.

2) The Propolis-Treated Group: Rats received propolis orally in a daily dose of 200 mg/kg b. w. for eight weeks.

3) MSG-Treated Group*:* This group included rats that were administrated MSG in a daily dose of 1g/kg b. w. for eight weeks.

4) The Protected Group*:* Animals of this group were first administrated propolis orally in a dose of 200 mg/kg b. w. daily for four weeks and secondly administrated daily oral doses of propolis (200 mg/kg b. w.) in association with MSG (1g/kg b. w.) for an additional four weeks.

5) The Therapeutic Group*:* Animals of this group were first provided with oral dose of MSG (1g/kg b. w.) daily for four weeks, then were treated orally with MSG (1g/kg b. w.) in association with propolis (200 mg/kg b. w.) for an additional four weeks.

Preparation of Tissue Samples:

At the end of experiment, animals from control and treated groups were sacrificed 24 h after the last dose of different administrations and then brains were rapidly excised, washed in saline to remove blood and other extraneous and dried on filter paper. Finally, samples of brains were kept in 10 % neutral buffered formalin solution for histological examination [24].

Histopathological Examination:

Brain specimens were dehydrated in ascending grades of ethyl alcohol (70 %, 90 %, and 100 %), cleared in xylene and impregnated and embedded in paraffin wax. Serial sections of 4-5 micrometers thick were obtained using a rotary microtome and stained with Harris's Haematoxylin and Eosin stain for general histological examination [25].

RESULTS

The brains of the rats administered propolis showed the same histological observations as in the brains of control animals. The brains of control and the propolis treated groups did not show any differences from normal structure of brain tissue. Examination of brain of the control and propolis administrated animals showed that normal structure of the brain seemed of regular histoarchitecture (Figures 1 and 2).

After 4 weeks of MSG administration the brains exhibited certain pathological changes including some perivascular cuffing, oedema and certain vacuolation of the neurons (Figure 3). At the end of the investigation, cross sections through

the brain treated with MSG for 8 weeks were, to great extent, damaged as designated by perivascular oedema and cuffing. Some blood vessels were congested. Vacuolation of some neurons were dominant in the field (Figures 4).
In the protective group, the brain sections indicated an obvious gliosis in the cerebrum. There was still some odematous changes and perivascular cuffing which were still persistent (Figure 5).
The brain sections of rats of the therapeutic group showed an active hyperaemia of the brain. Blood vessels were congested, and showed perivascular oedema. Some neurons exhibited a marked vacuolation (Figure 6).

DISCUSSION

Monosodium-L-glutamate (MSG) is a food additive, which serves as a useful flavor enhancer. It has been widely used for many years in human and livestock diets to promote consumption rates of a particular feed item [26]. Its use has increased throughout the world in recent years as flavoring in cooking [27]. Propolis is one of the natural products, which has more than 300 compounds of different groups, mainly phenolic acids and their esters, aldehydes and ketons have been identified from propolis [28]. The propolis-treated group showed normal histological brains architecture and almost similar to that demonstrated by the brains of control group**.** There seems to be a general agreement among experimental investigators that propolis is relatively nontoxic even at some higher doses [29], and its long term administration does not produce injury [14], which suggest its wider therapeutic index [30].

Administration of MSG resulted in certain pathological changes in the brains including some perivascular cuffing, oedema and certain vacuolation of the neurons and some blood vessels were congested. These findings are in agreement with the results observed by different authors, who also demonstrated perivascular cuffing [31, 32]; oedema [33, 34], vacuolation [35] and congestion of blood vessels as result of MSG administration [36]. However, the alterations in the brain tissues produced by MSG could be also due to either increase in the free radicals generation in the body [37] or due to the ability of MSG to induce oxidative damage in the brain, liver and kidney in experimental animals [10], which is considered another clarification for the deleterious effect of MSG in these organs. The excitotoxic effect of glutamate could be attributed to its ability to induce unlimited elevation of intracellular calcium resulting in activation of various enzymes that are responsible for cell death by various mechanisms [38]. These excitotoxic lesions in the lateral hypothalamus are neuronal loss and disruption of blood brain barrier [39].

Additionally, MSG is a neurotoxic agent i.e. causing damage to brain cells, retinal degeneration, many endocrine disorders, and renal damage [40].

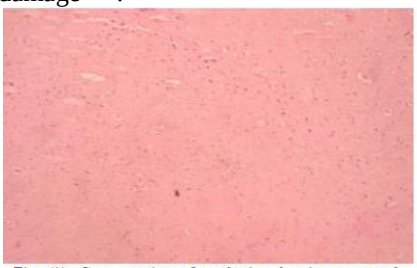

Fig. (1): Cross section of rat brain, showing a normal histological structure of the brain. (H & E, X 100).

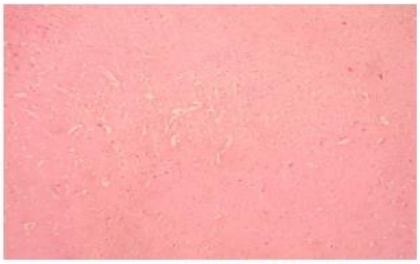

Fig. (2): Cross section of rat brain after treatment with propolis (200 mg/kg) for 4 weeks. It shows normal structure of the brain. No evidence of any pathological lesions (H & E, X 100).

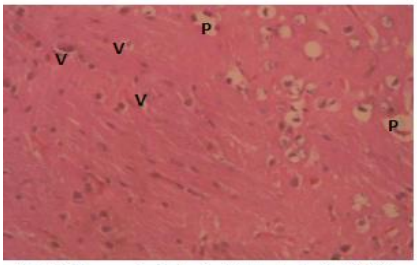

Fig. (3): Cross section in brain of rat treated with MSG for 4 weeks, exhibiting certain pathological changes. These include some perivascular cuffing (P) and certain vacuolation of the neurons (V). (H & E, X400).

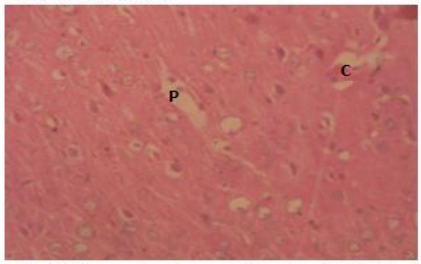

Fig. (4): Cross section from rat brain treated with MSG for 8 weeks, indicating a massive encephalatic oedema perivascular cuffing (P) with slight congestion of the blood vessels (C). (H & E, X400).

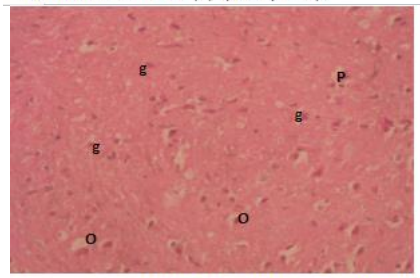

Fig. (5): Cross section through brain of rat from the protective group after 8 weeks, indicating an obvious gliosis (g) in the cerebrum. There is still some odematous changes (O) and perivascular cuffing (P) which are still persistent. (H & E, X 400).

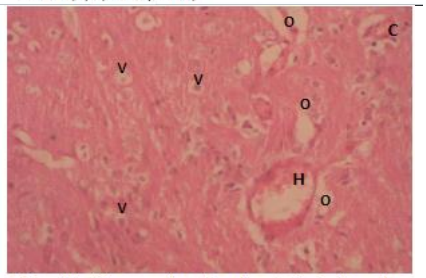

Fig. (6): Cross section through rat brain from the therapeutic group after 8 weeks, showing an active hyperaemia (H) of the brain. Blood vessels are congested (C), perivascular oedema (O). Some neurons exhibited a marked vacuolation (V). (H & E, X 400).

The protective group showed an obvious gliosis in the cerebrum. There is still some odematous changes and perivascular cuffing which were still persistent. The therapeutic group showed an active hyperaemia of the brain. Blood vessels were congested with perivascular oedema. Some neurons exhibited a marked vacuolation. These findings are in agreement with the results observed by different authors [29, 41]. On the other hand, this neurotoxicity was markedly mitigated by propolis treatment, and the ameliorative effects of treatments may be due to their antioxidant properties. Interestingly, propolis is rich in polyphenolic compounds, which are characterized by the highest antioxidant values and anti-inflammatory properties [42, 43].

CONCLUSION:

In conclusion, the results confirm the brain toxic effect of MSG and the moderate ameliorative effect of propolis.

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