Serrio biocamical efects of monosodium glutamate On wistar albino rats

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To
whom I belong soul
and all

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ABSTRACT

The present study was carried out to investigate the toxic effects of Monosodium glutamate (MSG) on Wistar albino rats.

Twenty rats were divided into four groups, five rats each, one group was left as control and the other groups were treated with three different levels of MSG (120, 240, 480 mg/kg body weight).

Clinical symptoms throughout the experimental period which lasted for 28 days were recorded.

Body weights, relative organs weight, some serum constituents and haematological changes beside histopathology of liver and brain were measured.

Higher levels of MSG showed paralysis in one leg, dizziness, hyperactivity and disorientation which reflected disturbances in the Central Nervous System.

The results indicated that MSG had no significant effects on body weight and relative weight of livers and kidneys, and haematological values.

Higher levels of MSG resulted in significant increase in activity of transeaminase enzymes and significant decrease in total protein concentration.

Histopathological examination of the liver showed degeneration and vaculation of hepatocytes beside dilation of sinosoids, while brain tissue showed congestion, oedema around the neurons, vaculation and neuronal necrosis.

This results prove that MSG has a toxic effects on liver and brain tissues. The toxicity increase whenever the rate of the salt increased too.

This study recommends that the necessity of avoiding the use of MSG as additive compress to human and animals` food because o f toxic effects of this salt.

خـلاصـة الأطروحة

أجريت هذه الدراسة لفحص التأثيرات السمية لملح قلوتاميت الصوديوم الأحادى على الفئران.

تم تقسيم 20 فأراً الى أربعة مجموعات، خمسة فئران بكل مجموعة.

أستخـدمت فى هـذه الدراسة ثلاثة مستويات من ملح قلوتاميت الصوديوم الأحادى (120، 240، 480 ملج/كجم من وزن الجسم).

تم قياس وزن الجسم والوزن النسبى للكبد والكلى، بعض مكونات السيرم، التغيرات فى مكونات الدم والتأثير المرضى على أنسجة الكبد والمخ بالاضافة الى ملاحظة الاعراض الاكلينيكية خلال فترة التجربة والتى إستمرت 28 يوماً.

زيادة نسبة ملح قلوتاميت الصوديوم الأحادى أدت الى ظهور أعراض إكلينيكية مثل شلل إحدى الأرجل، دوار، نشاط زائد و عدم المقدرة على تحديد الاتجاه ويعكس ذلك وجود إضطرابات فى الجهاز العصبى المركزى.

أثبتت النتائج أن ملح قلوتاميت الصوديوم الأحادى ليس له تأثير معنوى على وزن الجسم والوزن النسبى للكبد والكلى، كذلك لم يلاحظ أى تأثير معنوى على مكونات الدم.

زيادة نسبة ملح قلوتاميت الصوديوم الأحادى لها تأثير معنوى على زيادة نشاط إنزيمى القلوتاميت ترانساميناز وإنخفاض التركيز الكلى للبروتين بالسيرم.

أظهرت نتائج فحص الانسجة لقطاعات الكبد تنكس الخلايا الكبدية و تفجى وتوسع الجيبانيات بينما أظهرت قطاعات المخ إحتقان ووذمة حول العصبونات وتفجى ونخر العصبون.

هذه النتائج تثبت أن ملح قلوتاميت الصوديوم الأحادي له أثار سامه علي أنسجة الكبد والمخ وتزداد السمية مع زيادة نسبة الملح.

توصي هذه الدراسة بضرورة تجنب استخدام ملح قلوتاميت الصوديوم الأحادي كمادة مضافة لغذاء الإنسان والحيوان للآثار السامة لهذا الملح.

INTRODUCTION

Food additives used to enhance the taste of food or to alter the taste so as to mask disagreeable taste and magnify desired one.

They all have nothing to do with preserving food or protecting its integrity.

Monosodium glutamate (MSG), a food additive, is the sodium salt of the non-essential amino acid glutamic acid. Glutamic acid is one of the most abundant amino acids found in nature and exists both as free glutamate and bound with other amino acids into protein.

Glutamate is thus found in a wide variety of foods, and in its free form, where it has been shown to have a flavour enhancing effect.

As a result of its flavour enhancing effect, glutamate is often deliberately added to foods either as purified monosodium salt (MSG) or as a component of a mixture of amino acids and small peptides resulting from the acidic or enzymatic hydrolysis of proteins.

MSG has been implicated as the causative agent in the symptom complex known as Chinese restaurant syndrome

The purpose of this study is to determine if MSG has the potential to cause severe adverse reactions and excitotoxic effects on Wister albino rats.

Chapter One

Literature Review

1.1. Physical and Chemical Properties of MSG

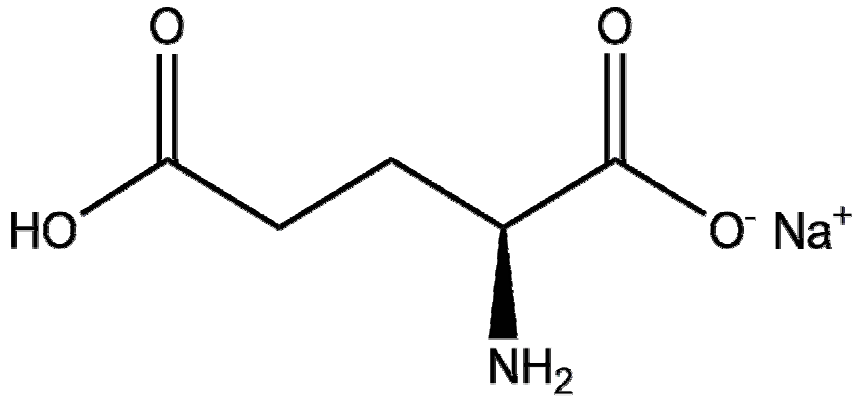

Fig [1]:- The Chemical Structure of Monosodium Glutamate

Monosodium glutamate ($C_5H_8NNaO_4$), European Union (EU) food additive code E621 commonly known as MSG is a sodium salt of glutamic acid. It is popularly marketed as flavor enhancer. In its pure form, it appears as a white crystalline powder, when dissolved in water or saliva it rapidly dissociates into sodium cations and glutamate anions (www.wikipedia.org). The chemical structure of MSG is shown in figure [1] above.

MSG has no texture or smell of its own (Singh, 2005).

MSG is readily soluble in water but sparingly soluble in ethanol. MSG is not hygroscopic and is considered quite stable, it does not change in appearance or quality during prolonged storage at room temperature. MSG does not decompose during normal food processing or cooking but in acidic conditions (pH 2.2-2.4) and at high temperatures it is partially dehydrated and converted to 5-pyrrolidone-2-carboxylate (Yamaguchi and Ninomiya, 1998).

An anionic form of MSG at physiological pH known as glutamate. Glutamate is one of many different amino acids, which are considered to be the building blocks of protein. Glutamate itself is regarded as one of the most important components in proteins (Michele *et al*, 1999).

More specifically, MSG is a manufactured glutamate to which a sodium ion has been attached. It is comprised of nothing more than water, sodium and glutamate (www.wikipedia.org).

1.2. Uses of MSG

The use of MSG in food began in the early 1900s as a component of flavor enhancer.

MSG is used to enhance the natural flavors of meat, poultry, seafood, snacks, soups and stews (Institute of Food Technologists, 1987a).

Food palatability increases with appropriate concentrations of MSG (Halpern, 2000).

The amount of glutamate used in foods is usually within the range of 0.1% to 0.8% of the food. This is similar to levels of naturally occurring glutamate found in traditional dishes. This mean that once the appropriate amount has been included in a recipe, adding more contribute little to flavor or may even be detrimental to the flavor balance of the dish (FAO/WHO, 1970[1]).

MSG basically causes the nerve cells to discharge an electrical impulse and that's the basis of its use as a flavor enhancer. It could also suppress or off undesirable flavors, bitterness and sourness and eliminated the tinny taste of canned food (Schwartz, 1988).

1.3. Sources of Glutamate

1.3.1. Natural Sources

Glutamate occurs naturally in every plant and animal as a part of enzymes and structural proteins. Free glutamate is also found in varying amounts in

many foods. Most proteins found in plants and animals contain from 5-25 % glutamate (Raiten *et al*, 1995).

Glutamate is found in abundance in both free and bound forms in all natural food stuffs; meat, poultry, fish, cheese, milk including human breast milk, tomatoes, mushrooms and many other vegetables (www.msg.org).

Of the twenty free amino acid in human breast milk, glutamate is the most abundant, accounting for >50% of the total free amino acid content (Rassin *et al*, 1978).

The free form of glutamate not linked to protein, in food increase its content during natural ripening of fruits (www.msg.org).

1. 3. 2. Commercial Sources

Although an extract of seaweed has been used by oriental cultures to enhance food flavor for over 1,000 years, it was not until 1910 that the essential component responsible for the flavor phenomenon was identified as glutamate and industrial production of glutamate and MSG commenced (Kizer *et al*, 1978).

From 1910 until 1956, the process underlying production of glutamate was one of extraction, a slow and costly method. In 1956, the Japanese succeeded in producing glutamate by means of fermentation; thus large-scale production of glutamate began (Nostrand, 1983).

MSG is created when protein is either partially or fully broken apart into its constituent amino acids, or glutamic acid is secreted from selected bacteria. A protein can be broken into its constituent amino acids in a number of ways (autolysis, hydrolysis, enzymolysis, and/or fermentation). In general, these processes are referred to as hydrolyzation of protein. When a protein is hydrolyzed, the amino acid chains in the protein are broken, and individual amino acids are freed. Acids, enzymes, and/or fermentation processes are used to hydrolyze protein (Leung and Foster, 1996).

The fermentation process was invented by Kyowa Hakko Kogyo in 1957.

A nonpathogenic species of Coryneform bacterium *Corynebacterium glutamicum* was originally isolated as an L-glutamate producing bacteria and is now used for industrial fermentative production of various amino acids (Takashi *et al*, 2001).

Mutation in it's *A* gene causes a growth defect and induce L-glutamate overproduction by *C-glutamicum.* [Figure 2] (Pamela and Richard, 1994).

Other organisms identified to produce L-glutamate are *Brevibacterium, Arithrobacter* and *Microbacterium.* (John and Bjorn, 1987).

The fermentation process begins with natural products such as molasses from sugar cane or sugar beets and food starch from tapioca or cereals with fermented in a controlled environment with a microorganism.

The crude glutamate produced in this process is then filtered, purified and converted by neutralization into MSG. After additional purification, crystallization, drying and sieving, MSG has the form of pure white crystals ready for packing and use (www.msg.org).

Combining specific amino acids, reducing sugars, animal or vegetable fats or oils, can also produce MSG (Lin, 1993).

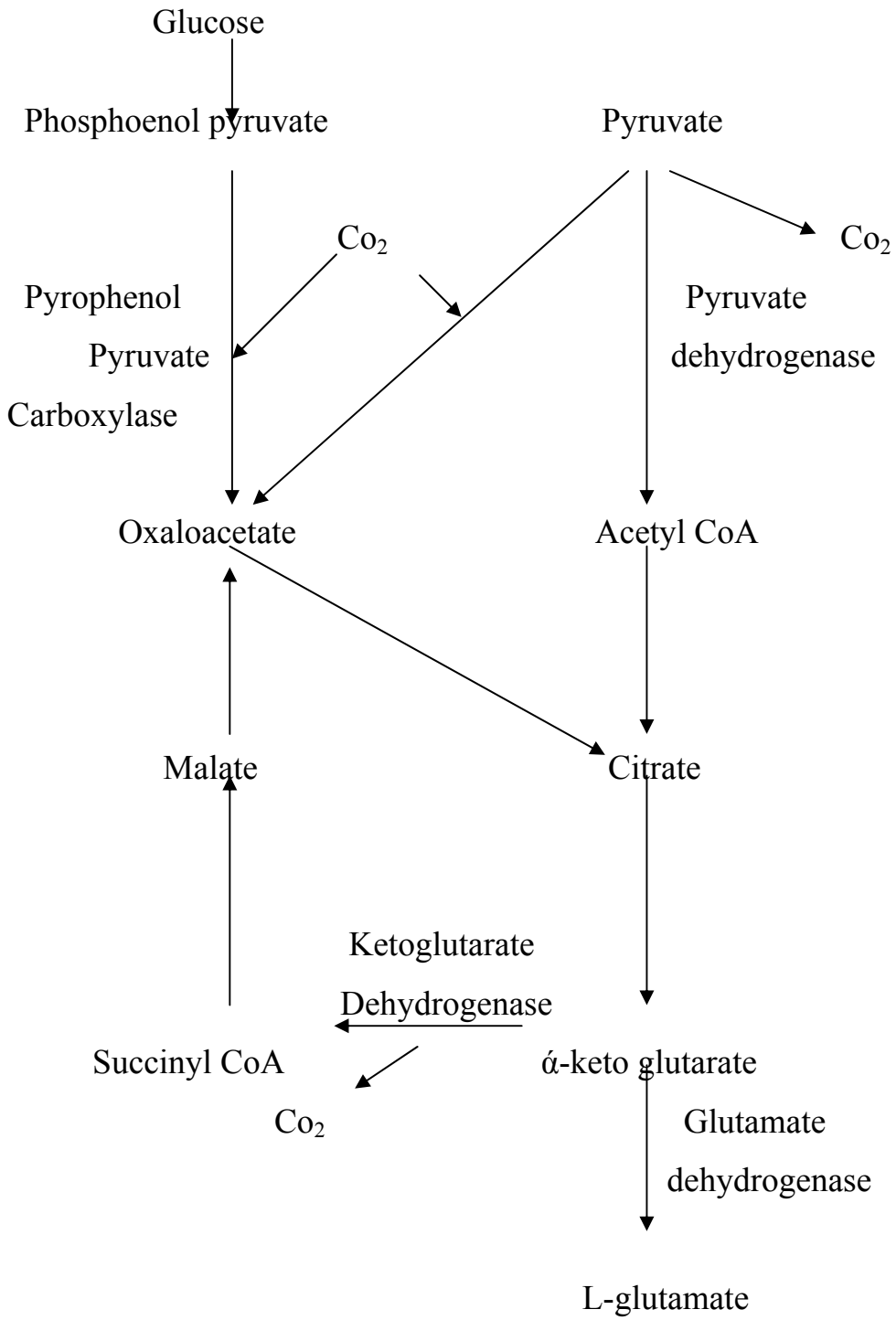

Fig [2]: - L-glutamate Production Pathway Reactions with *C. Glutamicum* Organism.

1.4. Flavoring Enhancement Properties of MSG

Flavors probably exert their effect by increasing the number of molecules that interact with receptors on chemosensory membranes in the nose and oral cavity and compensate for chemosensory losses. This intensification of chemosensory stimulation induces more salivation, produces greater stimulation of the olfactory and limbic system of the brain. Amplification of flavor and taste with MSG can improve food palatability and acceptance. MSG adds an additional taste but does not enhance any other tastes (Schiffman, 2000).

The use of MSG in food goes back to the oriental cooks who used seaweed called sea tangle to make a stock that added richness to the flavor of foods cooked in it. Professor Kikunae Ikeda discovered the link between the seaweed flavor improvement and glutamate in 1908. He demonstrated that the seaweed *Laminaria japonica* contained generous amounts of glutamate and that's the seaweed component responsible for food flavor enhancement (Institute of Food Technologests, 1980).

Glutamate is the key component in determining the flavor of protein-containing foods (FDA Backgrounder, 1995).

There are two types of glutamate; L- and D-isomers. L-glutamate is a natural component of protein, but D-glutamate is not. Flavoring action is recognized only in L-glutamate, and not in D- glutamate (Kuninaka *et al*, 1964).

When present in its free form, not bound together with other amino acids in protein, glutamate has a flavor enhancing effect in foods. When MSG is added to foods, it provides a flavoring function similar to the naturally occurring free glutamate (Institute of Food Technologists, 1987).

Only the free forms of glutamate have an effect on the glutamate receptors but when bound to other amino acids in a protein, it does not stimulate

glutamate receptors (Löliger, 2000).

MSG brings out the best natural flavors in food, working well in reduced-sodium and reduced-fat dishes and can reduce total sodium by 30-40 percent without influencing palatability (Yamaguchi and Takahashi, 1984).

Fuke and Shimizu (1993) indicated that MSG falls outside the region occupied by the four classic tastes of sweet, sour, salty and bitter.

This distinctive taste is known as *umami*, a word coined by the Japanese to describe the taste imparted by glutamate. Westerners often describe this flavor as savory, broth-like or meaty (Yamaguchi, 1987).

The tongue is sensitive to five flavors, salt, sweet, bitter, sour, and *umami* (Schiffman, 2000).

The substances which constitute the umami taste can be divided in two main groups: one is the amino acid group, represented by monosodium glutamate and the other is the 5'-nucleotid group, represented by inosine 5'-monophosphate (IMP) and guanosine mono phosphate (GMP) and their derivatives (Papi, 2003).

MSG, with or without 5'-ribonucleotides, likely exerts its effect by adding another taste quality to the food which improves palatability (Bellisle *et al*, 1991).

Neither MSG nor 5'-ribonucleotides appear to exert their effect by altering the perceived intensity of other components of food or altered the intensities of salts, sweeteners, amino acids or bitter compounds (Schiffman, 2000).

The basic sensory function of MSG is attributed to its ability to enhance the presence of other taste-active compounds. Foods containing MSG have a typical salty taste, because it contains 12.3 % sodium. The detection threshold for MSG is 6.25×10^{-4} mol/L, which interestingly is higher than

for bitterness or sourness, and lower than that for sweetness and about equal to that for saltiness. In general, the usage level of MSG in savory foods is approximately one tenth that of salt; thus the sodium contribution of MSG is roughly one thirtieth of the total added sodium. By adding MSG appropriately, the sodium chloride addition could be reduced by 30-40 % while maintaining the same perception of saltiness. Results of taste panel studies on processed foods indicate that an MSG level of 0.2-0.8 % of food by weight optimally enhances the natural food flavor (Löliger, 2000).

Bellisle *et al*. (1996) stated that addition of MSG to nutritionally valuable foods would represent a mean to selective develop preference for food or to enhance its intake without increasing total energy intake.

The optimal palatability concentration for MSG is between 0.2-0.8% and its use tends to be self-limiting as over-use decrease palatability. The largest palatable dose for humans is about 60mg/kg body weight (Walker and Lupien, 2000)

1.5. Metabolism and Synthesis of Glutamate

Glutamate performs essential roles in intermediary metabolism and present in large amounts in the organs and tissues of the body. The daily turn over of glutamate in the adult human has been estimated as 4800m/h (Munro, 1979).

Human brain is the only tissue that has the highest content of glutamate (Hardman *et al*, 2001).

The majority of the glutamate used by the brain is derived from local synthesis from glutamine and tricarboxylic acid cycle (TCA) intermediates and a considerable fraction is also derived from the recycling of brain protein (Smith, 2000).

As part of protein digestion, protein is broken down into its constituent's amino acids. The ingested protein is hydrolyzed in the stomach and lower intestine through the action of hydrochloric acid and enzymes. In the

human body, glutamate can be formed from ingested protein (Freedland and Briggs., 1977).

The body controls the amount of glutamate converted from protein in this way and dispose of the waste, human do not store glutamate as such (Burgess, 1988).

Glutamate plays a central role in transamination reaction in which amino acids funnel their amino group to α-ketoglutarate resulting in glutamate formation. Glutamate therefore acts as an acceptor of amino group from other amino acids and further undergoes either oxidative deamination in liver or is used as amino group donor in synthesis on non-essential amino acids. Glutamate thus undergoes either transamination, resulting in formation of α-ketoglutarate, which enters TCA cycle and aspartate which enters urea cycle or oxidative deamination in the liver by the enzyme glutamate dehydrogenase resulting in the liberation of free ammonia which enters urea cycle (Chanda *et al*, 2005).

Glutamate is absorbed from the gut by an active transport system specific for amino acids (Schultz *et al*, 1970).

During intestinal absorption, a large proportion of glutamate is transaminated and consequently alanine levels in portal blood are elevated. If large amounts of glutamate are ingested, portal glutamate levels increase (Stegink, 1984). This elevation results in increased hepatic metabolism of glutamate, leading to release of glucose, lactate, glutamine and other amino acids into systemic circulation (Stegink, 1983).

Glutamic acid in dietary protein, together with endogenous protein secreted into the gut, is digested to free amino acids and small peptides, both of which are absorbed into mucosal cells where peptides are hydrolyzed to free amino acids and some of glutamate is metabolized. Excess glutamate appears in the portal blood where it is metabolized by the liver. As a

consequence of the rapid metabolism of glutamate in intestinal mucosal cells, with any excess glutamate being metabolized by the liver, systemic plasma levels are typically low, even after ingestion of large amounts of dietary protein (Munro, 1979).

A number of early studies with dogs (Neame and Wiseman, 1958), and later, studies conducted in rats (Windmueller, 1982), demonstrated that the vast majority of dietary glutamate is metabolized by the gastrointestinal tract. In fact, very little dietary glutamate enters either the systemic or the portal blood supply. Young and Ajami *et al*. (2000) indicated that glutamate is almost exclusively utilized by the intestinal tissues.

Moreovere, Olney (1975) stated that there is differences between ingesting a free amino acid, and ingesting of amino acid bound in protein, in the former case, absorption of the entire amino acid load is immediate and this leads to much higher peaking of the amino acid level in the blood than if a similar amount were released slowly by over a matter of hours by the digestive process. There is evidence that the gut transaminate glutamate to alanine, an amino acid that does not have excitotoxic potential and this may represent a protective mechanism to prevent elevated blood levels of this neurotoxic amino acid.

Hence, bound glutamate found naturally in foods, is less dangerous, and can be utilized by the tissues before toxic concentrations can be built (Blaylock, 1998).

However, composition of the dosing vehicle as well as the conditions of administration of the dose has significant impact on changes in circulating glutamate in response to oral ingestion (Raiten et al, 1995).

The process of dietary glutamate utilization by the intestinal tract has recently been extensively studied. The results showed that 95% of dietary glutamate presented to the mucosa was metabolized in first pass and that of this, 50% appeared as portal CO_2, with lesser amounts as lactate and

alanine. This indicates that glutamate is the single largest contributor to intestinal energy generation. The studies also indicated that about 10% of dietary glutamate is incorporated into mucosal protein synthesis, with the remainder being used for the synthesis of proline, arginine and glutathione. In fact, all three substances proline, arginine and glutathione are derived almost exclusively from dietary glutamate, rather than the vast *in vivo* pool of glutamate (Reeds *et al*, 2000).

Glutamate transportors in skeletal muscles and heart appear to play a role in the control of the steady-state concentration of amino acids in the intracellular space probably through osmatic signaling mechanisms to regulate whole body protein metabolism (Rennie *et al*, 1996).

The fate of ingested MSG in some cases does not come to rest in the plasma as elevated plasma glutamate and from there to be excreted by the liver. Rather it would appear that the fate of ingested processed free Glutamic acid might be dispatch to any glutamate receptors available and create adverse or toxic reactions if the peripheral glutamate receptors are weak, crippled, diseased or otherwise unhealthy (www.truthinlabeling.org).

1. 6. Adverse Reactions to MSG

In 1968, a letter was published in the New England Journal of Medicine describing a syndrome, which began 15 to 30 minutes after eating Chinese food containing MSG, and lasted about 2 hours with no lasting effects. The symptoms were described as numbness at the back of the neck gradually radiating to both arms and the back, general weakness and palpitation (Kwok, 1968).

Schaumburg *et al*. (1969) reported results of studies they had under taken. This times both headache and chest pains were added to the symptoms list.

Later on, there have been reports of tachycardia (Gann, 1977), hyperactive or hysterical activity in children (Asnes, 1980), paraesthesiae of hands and

feet (Freed and Carter, 1982), severe burning headache, severe upper abdominal pain, pressure accompanied by diaphoresis and a burning sensation in the chest (Ratner *et al*, 1984), angio-oedema (Squire, 1987) and a hypertensive reaction in the form of vascular headache (Pohl *et al*, 1988).

Studies completed in the 1970s in United States declared that at least 25 percent of the population reacts to MSG in processed food (Reif-Lehrer, 1977).

Reif-Lehrer and Stemmermann (1975) stated that children react to ingestion of MSG and describing CNS symptoms similar to adults with almost the same degree of prevalence. She discussed the fact that glutamic acid has been reported to cause convulsive disorders in animals.

Subsequently, Andermann commented on a possible relationship between glutamic acid and essential tremor (Andermann *et al*, 1975).

Colman (1978) stated two cases of psychiatric reactions to monosodium glutamate.

Ratner *et al*. (1984) stated that the initial diagnoses in seven patients, whose complaints were eventually resolved as MSG sensitivity, were migraine, myocardial infarction, brain tumor, neurosis, functional colitis, and depression.

Schwartz (1988) reported that MSG-reactions range from mild and transitory to debilitating and/or life threatening, including skin rash, simple headache, nausea/vomiting, asthma-like symptoms, migraine headache, tachycardia, panic attack, anaphylactic shock, seizures and depression.

In 1995, the Federation of American Societies for Experimental Biology (FASEB), who had been commissioned by the United States Food and Drug Administration (FDA) to undertake a review of reported adverse reactions to MSG, reported that the following symptoms are considered

representative of the acute, temporary, and self-limited reactions to oral ingestion of MSG (FASEB, 1995):

- Burning sensations in the back of the neck, forearms, and chest.

- Facial pressure/tightness.

- Chest pain.

- Headache.

- Nausea.

- Palpitation.

- Numbness in back of neck, radiating to arms and back.

- Tingling, warmth, weakness in face, temples, upper back, neck and arms

- Drowsiness.

- Weakness.

In some recently conducted studies, the most frequently reported symptoms were headache, numbness/tingling, flushing, muscle tightness, and generalised weakness (Yang *et al,* 1997 and Geha *et al*, 2000).

Ingestion of processed free glutamic acid causes adverse reactions in susceptible individuals. The fairly recent discovery of glutamate receptors in many locations outside the central nervous system suggests that the readily observable toxic effects of processed free glutamic acid, referred to as adverse reactions, are facilitated by glutamate receptors in the mouth, lungs, intestines, and muscle (Gill *et al*, 2000).

Symptoms are reported to occur at doses like 1.5 and 12g of glutamate after 15-25 minutes. The threshold range for intravenous dose is 25-125 mg for minimum symptoms to occur after 17-20 seconds. At supra-threshold dose, tightness, pressure over malar, numbness, burning sensation over chest, forearms, abdomen, and thighs have been reported

(www.truthinlabeling.org).

Onset time for the adverse reactions being considered as possible reactions to MSG was 10-25 minutes with duration of 45 minutes to 2 hours.

1.7. MSG Excitotoxicity

There is increasing scientific evidence, however, that taste cells on the tongue are not only the things that taste enhancers stimulate. When neurons in the brain are exposed to these substances, they become very excited and fire their impulses rapidly until they reach a state of extreme exhaustion. Several hours later these neurons suddenly die, as if the cells were excited to death (Shambaugh, 1996).

The toxicity of glutamate was observed in 1957 when the feeding of MSG to newborn mice destroyed the neurons in the inner layers of the retina (Lucas and Newhouse, 1957).

Olney (1969) discovered that the phenomenon wasn't restricted to the retina but occurred throughout the brain and coined the term excitotoxicity.

Excitotoxicity is the pathological process by which neurons are damaged and killed by over activations of receptors for glutamate (Manev *et al*, 1989).

Glutamate is required for normal brain function while excess amount leads to neuronal death due to the destructive effect mediated by glutamate receptors (Shaw, 1999).

Excitotoxicity can occur from substances produced within the body. Glutamate is a prime example of an excitotoxin in the brain, and it is paradoxically also the major excitatory neurotransmitter in the mammalian central nervous system (CNS) (Temple *et al*, 2001).

Glutamate ability to destroy neurons is mediated by the interaction with N-methyl-D-aspartate (NMDA) receptors which induces intracellular calcium increase, free radical generation, and activation of proteases, phospholipases and endonucleases, and the transcriptional activity of

apoptotic programmers (Pelligrini *et al*, 1997).

During normal conditions, glutamate concentration can be increased up to 1mM in the synaptic cleft, which is rapidly decreased in the lapse of milliseconds. When glutamate concentration around the synaptic cleft cannot be decreased or reaches higher levels, the neuron kills itself by apoptosis process (Kanded *et al*, 2000).

At chemical synapses, glutamate is stored in vesicles. Nerve impulses trigger release of glutamate from the pre-synaptic cells. In the opposing post-synaptic cells, glutamate receptors bind glutamate and activated (www.wikipedia.org).

Shigeri *et al.* (2004) stated that glutamate transporters are found in neuronal and glial membranes. They rapidly remove glutamate from the extracellular space. In brain injury or disease, they can work in reverse and excess glutamate can accumulate outside cells.

Normally excess glutamate bumped back in the glial cells surrounding the neurons. However, when cells are exposed to excessive amount of glutamate, the neuron cells die. Glutamate opens the calcium channel in the neuron so that calcium can move into the cell. Magnesium normally blocks the calcium channel from opening. Glutamate removes this block and opens the calcium channel, a normal reaction. However, when glutamate levels become even slightly excessive, the calcium channel in some neural cells can get stuck open, leading to destruction of those cells and adjacent cells. Not every nearby brain cell is affected, only the cells with glutamate receptors (www.holistimed.com).

Sometimes the cells are damaged without being killed because of the particular functions of the brain areas where these cells are located. The mode of action of excitotoxins on an individual neuron has been shown to weaken the membrane that surrounds each living cell. While exciting the neurons to fire repeatedly, the excitotoxins allows calcium to enter the cell

through its membrane. This causes the production of free oxygen radicals, which are believed to be the central cause for every injury and disease (Blaylock, 1994).

Glutamate is used as a neurotransmitter by glutamate-type neurons. Surrounding these neurons are helper cells, called asrtocytes, which regulate the concentration of glutamate by absorbing any excess and converting it into glutamine. If the asrtocytes are deprived of glucose or oxygen they become energy depleted and spill glutamate, killing or damaging these neurons in the absence of any excess dietary glutamate. When excess glutamate is present, it is one hundred times more toxic if the brain is also deprived of glucose. Since glutamate occurs naturally in foods, the brain has a second mechanism to help prevent excessive glutamate levels, the blood-brain barrier, which has an increased capability to transport beneficial substances such as glucose and exclude detrimental ones such as glutamate (Blayloch, 1994).

Excitotoxins play a critical role in the development of several neurological disorders including migraines, seizures, infections, abnormal neural development, certain endocrine disorders, neuropsychiatric disorders, learning disorders in children, episodic violence, hepatic encephalopathy, specific types of obesity, and especially the neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), parkinson's disease and alzheimer's disease (Ikonomidou and Turski, 1995).

1. 8. MSG and Central Nervous System

Research over the course of the last four decades has demonstrated that in addition to its role as a building block of protein, glutamic acid serve as a neurotransmitter vital to the transmission of nerve impulses in many parts of the (CNS) (Shank and Aprison, 1988).

Glutamate is the major excitatory transmitter within the brain, mediating fast synaptic transmission and is active in perhaps one third of (CNS)

synapses (Watkins and Evans, 1981).

It has also been demonstrated that under certain circumstances, glutamic acid, along with other acidic amino acids, functions as a neurotoxin, causing neuron degeneration and neuroendocrine disorders in a variety of laboratory animals (Garattini, 1979).

Glutamate is widely available in (CNS). It is highly concentrated in those regions of the brain that are essential in cognitive processes mediation, eg. cerebral cortex, hippocampus gyrus dentatus and striatum, indicating an important role of this amino acid in higher cognitive functions including memory (Cotman *et al*, 1987).

Cellular bodies of hypothalamic secretory neurons are situated in the areas protected by the blood-brain barrier, while their terminal axons are localized in eminentia mediana which lacks blood-brain barrier (Peruzzo *et al*, 2000). That is the reason why the eminentia mediana region which accepts the axonal terminals from the nearby arcuate nucleus and other hypothalamic secretory neurons is most sensitive to glutamate exposure.

Moreover, the fenestrated capillary endothelium of the eminentia mediana makes it available to plasma amino acids; so that the initial glutamate induced neuronal damage could be the result of circulating level of these acids rather than the cerebro-ventricular pool. The arcuate nucleus-eminentia mediana region in the early postnatal period in rodents was an often used as a model in the studies of MSG induced neurotoxcicity due to its marked sensitivity, consistent cytoarchitecture and prominent anatomic site (Takasaki, 1978).

Though the tanycyte modified astroglial cells forming tight connections and making up the inner blood-brain barrier surface network is already established in neonatal mice, arcuate and other neuronal axons is grown into eminentia mediana during the first 25 days of neonatal life (Eurenius and Jarskar, 1971).

Large doses of MSG administrated to immature animals do not cause evident tanycyte or eminentia mediana terminal axons damage, but the arcuate nucleus neurons are significantly damaged (Holzwarth-McBride *et a*, 1976).

This nucleus is the production site of numerous stimulatory and inhibitory hormones, that is why the disturbance of its function in neonatal period by MSG treatment leads to numerous endocrine and metabolic disorders and altered behaviour in the adult age (Klingberg *et al*, 1987 and Olney, 1969).

Neurotoxic effects of MSG induce growth retardation, obesity and sterility, reduction of growth hormone, gonadal steroid and thyroid hormone levels (Bake *et al*, 1978).

Chapter Two
Materials and Methods

2. 1. Materials and Experimental Design

2. 1. 1. Monosodium Glutamate

Monosodium glutamate in a white granular crystalline form was brought from Omdurman Central Market. Sudan.

2. 1. 2. Animals

Twenty healthy *Wistar albino* rats of both sexes were supplied by breeding unit at Central Veterinary Research Laboratory. Soba.

They were housed under standard conditions and have free access to water and standard diet.

They were left for two weeks as an adaptation period.

2. 1. 3. Experimental Design

At the end of the adaptation period the rats were divided randomly into four groups, five rats each. Group A was left as a control, while group B, C and D were orally administrated with MSG using gastric tube daily at concentrations of 120, 240 and 480 mg/kg body weight respectively for 28 days.

2. 1. 4. Parameters

Clinical signs and mortality were recorded. Blood samples were obtained for haematological investigation included red blood cells (RBCs) count, white blood cells (WBCs) count, packed cell volume (PCV) and haemoglobin (Hb) concentration.

Serum investigation included total protein concentration (TP), alanine amino transferase (ALT) activity, aspartate amino transferase (AST) activity and alkaline phosphatase (ALP) activity.

Slices from liver and brain were collected and fixed in 10% neutral buffered formalin for histopathological investigation.

2. 2. Methods

2. 2. 1. Haematological Methods

Blood samples were collected by puncturing the rat's retro orbital plexus with heparinized capillary tube (SUPE-PIRO-GERMAN code N-4361) into

dry clean tube containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant according to Waynforth (1980).

2. 2. 1. 1. Red Blood Cells (RBCs) Count

RBCs were counted using improved Neubaur haemocytometer (Hawksly and son Ltd, England). Haymem`s solution was used as diluent (Sodium Chloride 1.0g, Sodium Sulphate 0.5g, Mercuric Chloride 0.5g and made up to 200ml with distilled water. RBCs were expressed in million/mm^3 blood.

2. 2. 1. 2. White Blood Cells (WBCs) Count

WBCs were counted using improved haemocytometer (Hawksly and son Ltd, England). Turk's solution was used as diluent. WBCs were expressed in thaousand/mm^3 blood.

2. 2. 1. 3. Packed Cell Volume (PCV)

PCV was measured using haematocrit method. Blood samples were placed in the capillary haematocrit tube and centrifuged using haematocrit centrifuge (Hawksly and son Ltd, England).

The PCV percent was read off on the scaling instrument provided with microhaematocrit.

2. 2. 1. 4. Haemoglobin (Hb) Concentration

The determination of Hb concentration was based on the conversion of Hb to cyanomethaemoglbin by means of Drabkin's solution (Potassium cyanide 0.5g, Potassium ferricyanide 0.2g and Potassiun hydrogen orthro phosphate 0.14g in liter distilled water). The colored solution was read with spectrophotometer (JENWAY 6305 UV/VIS) at wave length 540 nm and compared against standard Hb (cromatest).

Haemoglobin concentration was expressed as follow:

$$\text{Hb (g/dl)} = \frac{\text{Tested Sample} \times 15}{\text{Standard}}$$

Where 15 was standard concentration.

2. 2. 2. Serum Biochemical Methods

Blood samples were collected in a similar procedure as for haematology into dry clean tubes without anticoagulant and allowed to clot at room temperature for 30 minutes then centrifuged (Hittich EBA35) at 3000 r. p. m for 5 minutes. Sera were separated and stored at -20 C° till analyses.

2. 2. 2. 1. Total Protein (TP) Concentration

The determination of TP concentration was done according to Biuret method (Reinhold, 1953).

The principal of this method is based on the reaction of peptide bond with cupric ion in alkaline media, the Biuret solution (Sodium potassium tartarate 9.0g in 500ml 0.2N sodium hydroxide, cupric sulphae 3.0g, potassium iodine 5.0g and made volume to one liter with 0.2N sodium hydroxide). This result in formation of colored complex.

The colored solution was read with spectrophotometer (JENWAY 6305 UV/VIS) at a wave length of 540nm.

The total protein concentration was calculated as follow:

$$\text{TP (g/dl)} = \frac{\text{Tested Sample} \times 6}{\text{Standard Protein}}$$

Where 6 was standard concentration.

2. 2. 2. 2. Transaminase Enzymes

The transaminase enzymes, aspartate transaminase (AST) and alanine transaminase (ALT) catalyze the transfer of the amino group of glutamic acid to oxaloacetic acid and pyruvic acid in reversible reactions.

The transaminases activity is proportional to the amount of oxaloacetate or pyruvate formed over a definite period of time and is measured by a reaction with 2,4-dinnitrophenyl hydrazine (DNPH) in alkaline solution.

The determination of transaminases activity was done according to colorimetric method (Reitman and Frankel, 1957).

2. 2. 2. 2. 1. Alanine transaminase (ALT) Activity

0.5 ml ALT substrate (Alanine 200mmol/l, Ketoglutarate 2mmol/l) was incubated for five minutes at 37˚C, 100µl of sera were added, mixed and incubated for 30 minutes, then 0.5ml 2,4-dinitrophenyl hydrazine (DNPH) 1mmol/l were added, mixed and allow to stand for 20 minutes at room temperature. An auxiliary reagent (NaOHO24N 5.0ml) were then added, mixed and let to stand for 15minutes at room temperature.

The optical density was read at 505nm against water blank.

ALT values were expressed in U/L.

2.2.2.2.2. Aspartate transaminase (AST) Activity

Similar procedures as in ALT were carried out except that the solution after the addition of serum was incubated for 60 minutes.

AST values were expressed in U/L.

2. 2. 2. 3. Alkaline Phosphatase (ALP) Activity

It is an optimized method according to the recommendation of Chemie (1972).

In alkaline medium serum alkaline phosphatase splits p-nitro-phenyl phosphatase, in the presence of Mg^{+2}, into p-nitrophenol and phosphate at the pH of the reaction, p-nitrophenyl was coloured yellow, and the optical density measured in a spectrophotometer (Jenway 6305 UV/VIS) at a wave length of 405 nm.

p-nitrophenyl phosphatase + $H_2o \rightarrow$ phosphate + p-nitrophenol

U/I = 2760 × A 405 nm/min.

(A = the mean of sample absorbance reading)

2. 2. 2. 4. Histopathological Methods

Slices from brain and kidney were collected and fixed in 10% neutral buffered formalin (Sodium hydrogen 6.5 g/l and Sodium dihydrogen 4.0g/l), then embedded in paraffin wax sectioned at 5µm and stained by Hematoxyline and Eosin (H&E) according to Culling (1974).

2. 2. 2. 5. Statistical Analysis

Data were analyzed statistically by Statistical Package for Social Scientists (SPSS) program, v.13 (2002).

Chapter Three

The Results

3. 1. Clinical Signs and Mortality

There were no clinical signs observed in control group. However all groups treated with MSG showed signs of shiver, dizziness and disorintation at day 14 post treatment. At day 17 there were hyperactivity and hysterical signs.

In group (B) which received 120mg/kg Bwt two rats showed paralysis of one leg at day 19 [fig3]. By day 26 paralysis of one leg appeared in most of the rats in group (C) and group (D) which received 240 and 480 mg/kg Bwt respectively.

Fig [3]: Paralysis in one leg in rat received 480 mg/kg body weight.

3. 2. Body Weight and Relative Organs Weight

There were no significant differences observed in body weights between control rats and treated ones [fig 4]. However the rate of weight gain in control rats was 26.4% compared to the group treated with 120mg/kg Bwt

which was 30.6%. In group treated with 240 and 480mg/kg Bwt the rate of gain was 31.5% and 32.1% respectively.

There were no significant differences observed in relative weights of liver and kidney between control rats and those treated with MSG [fig 5].

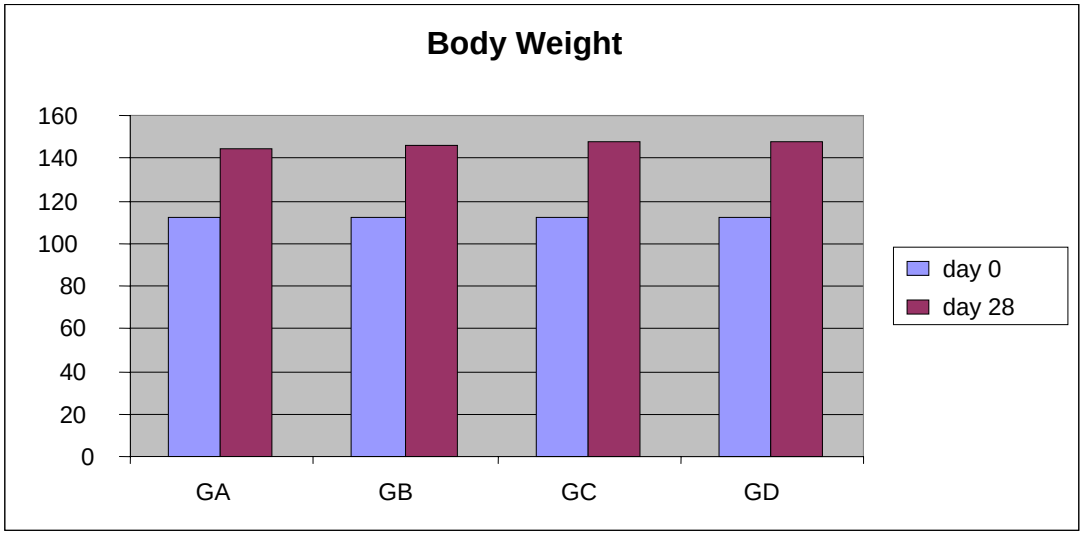

Fig [4]: The means body weight (mg) in rats treated with various levels of MSG.

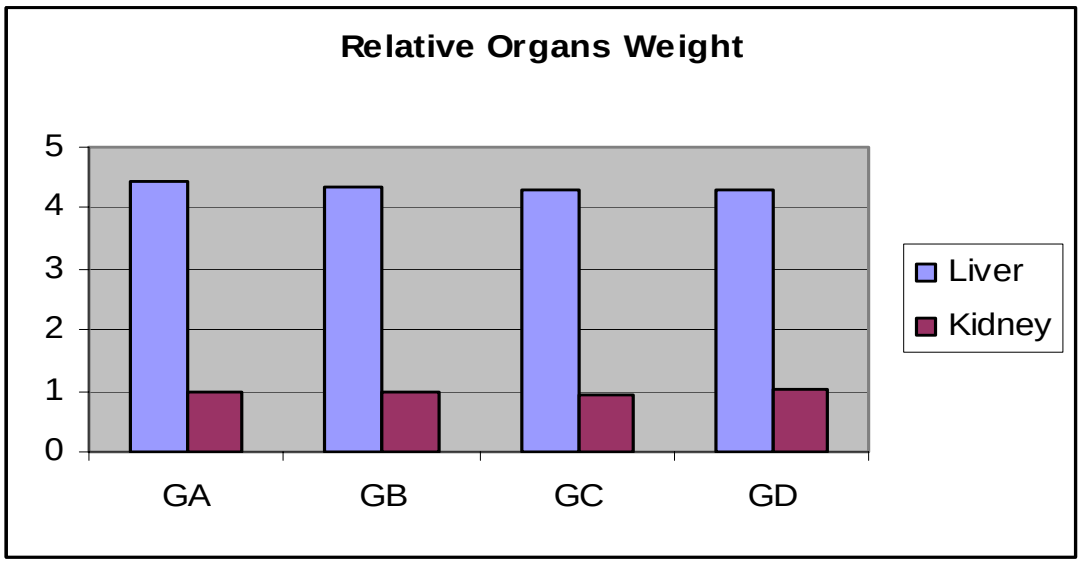

Fig [5]: The relative organs weight (mg) in rats treated with various levels of MSG.

3. 3. The Haematological Findings

There were no significant differences in total white blood cell count between the control and treated groups [fig 6]. The total red blood cell count was high in the groups treated with 480 mg/kg Bwt than the control but is not significant [fig 7].

The haemoglobin concentration was always similar between the control and treated groups [fig 8].

The packed cell volume was not significantly different between the control and treated groups [fig 9].

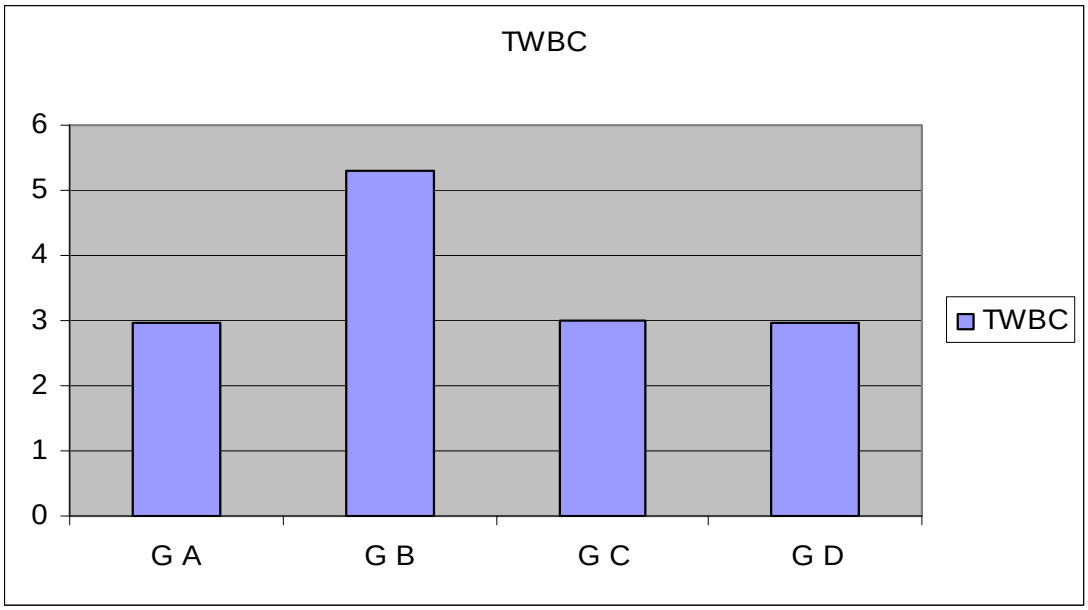

Fig [6]: Changes in total white blood cells count in rats treated with various levels of MSG.

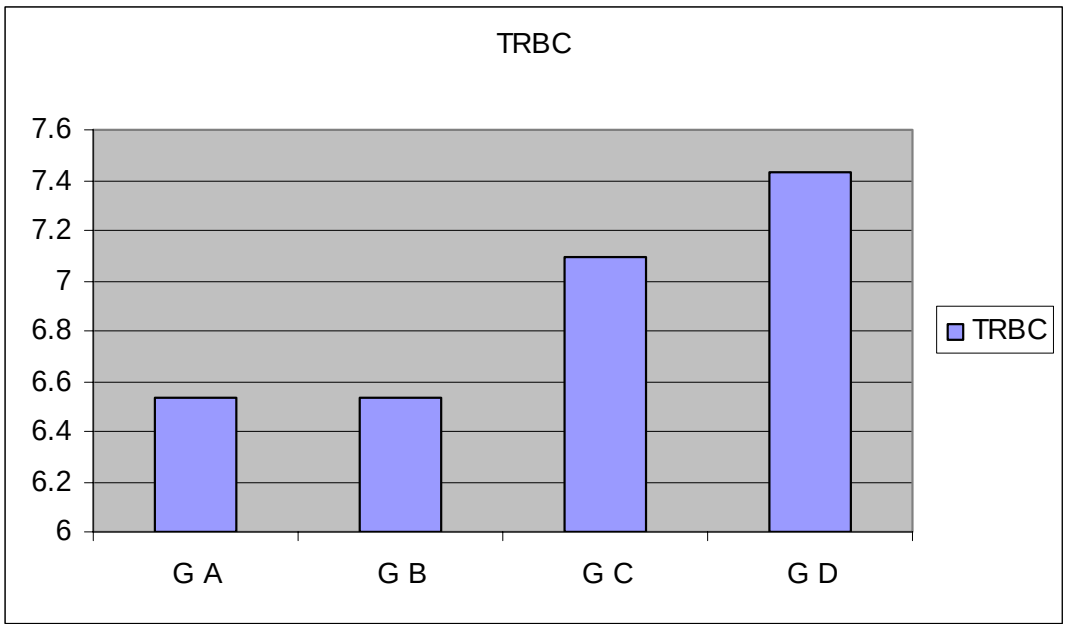

Fig [7]: Changes in total red blood cells count in rats treated with various levels of MSG.

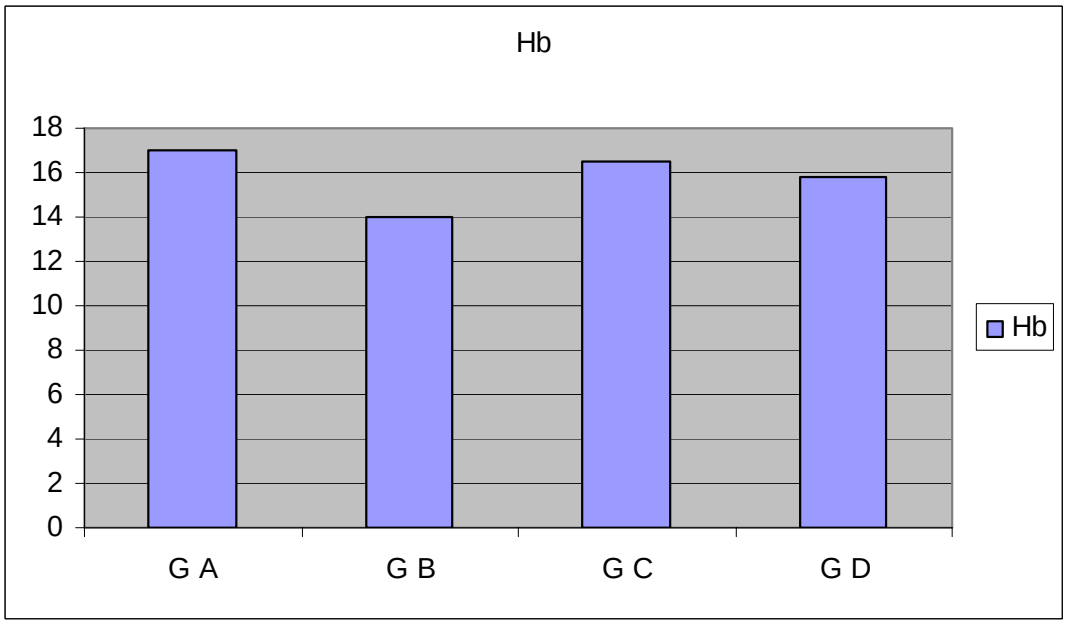

Fig [8]: Changes in haemoglobin concentration in rats treated with various levels of MSG.

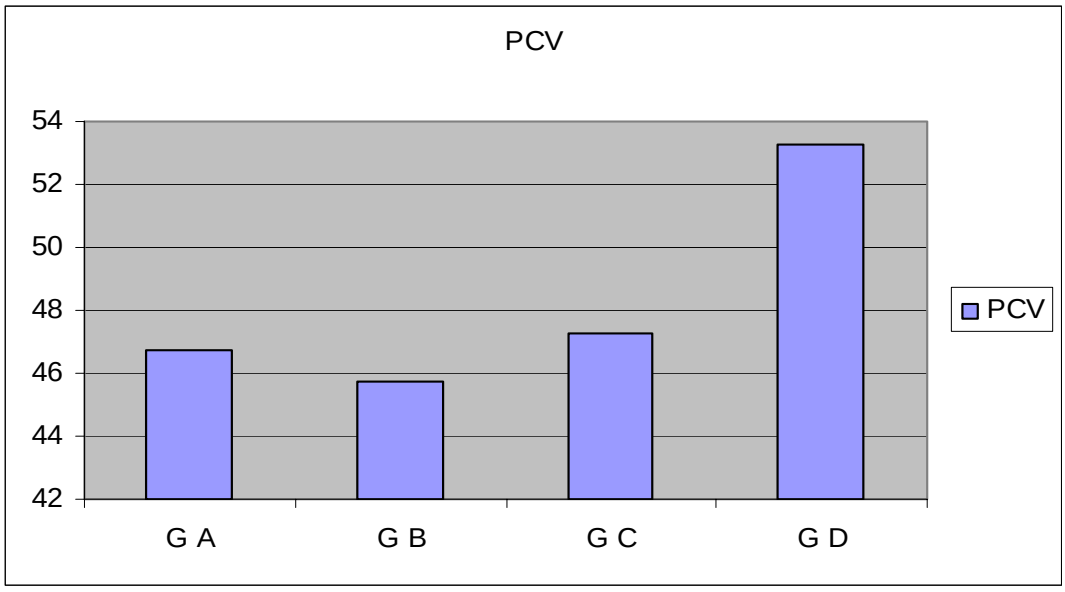

Fig [9]: Changes in packed cells volume values in rats treated with various levels of MSG.

3. 4 Changes in serum constituents

The ALT activity was increased significantly in all groups treated with MSG [fig 10].

There were significant differences in AST activity between the control and the treated groups. However, AST was increased significantly between the control group and the treated ones [fig 11].

The ALP activity increased significantly between the control and the treated groups [fig 12].

There were significant decreases in total protein concentrations between the control group and the treated groups [Fig 13].

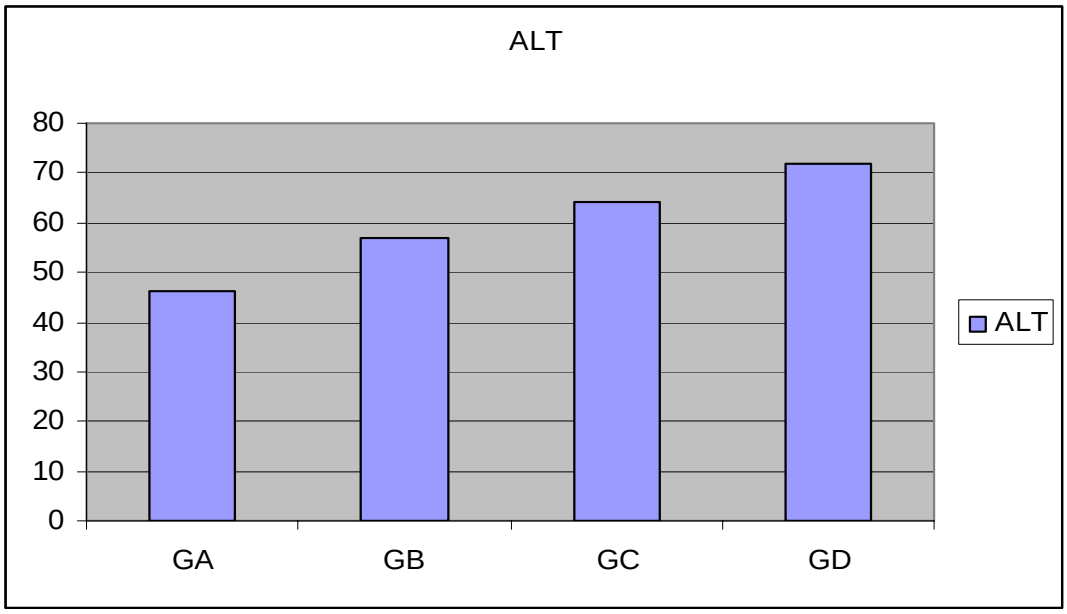

Fig [10]: Changes in ALT activity in rats treated with various levels of MSG.

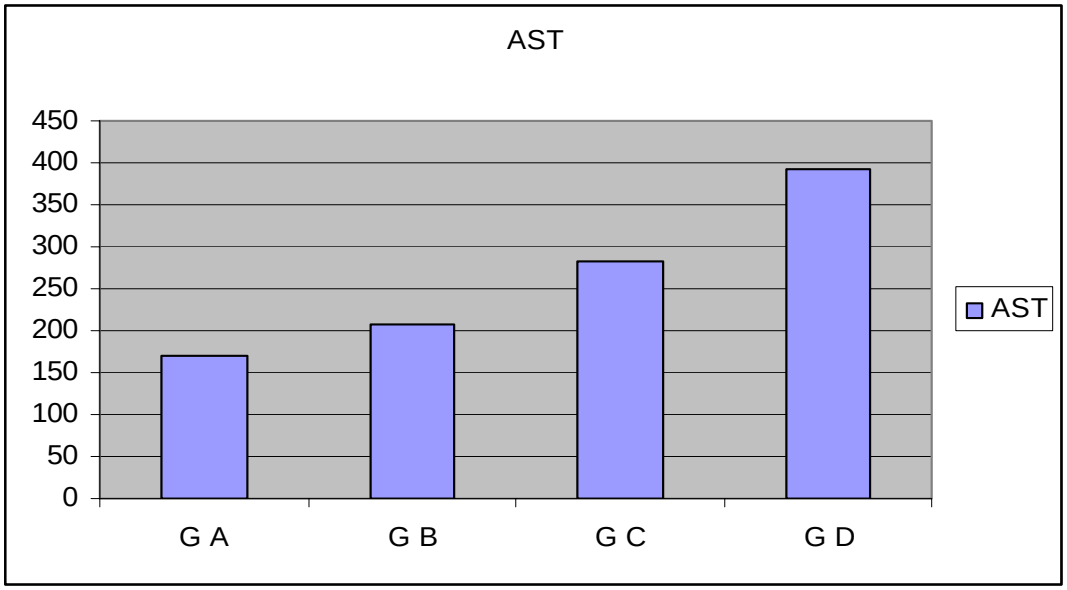

Fig [11]: Changes in AST activity in rats treated with various levels of MSG.

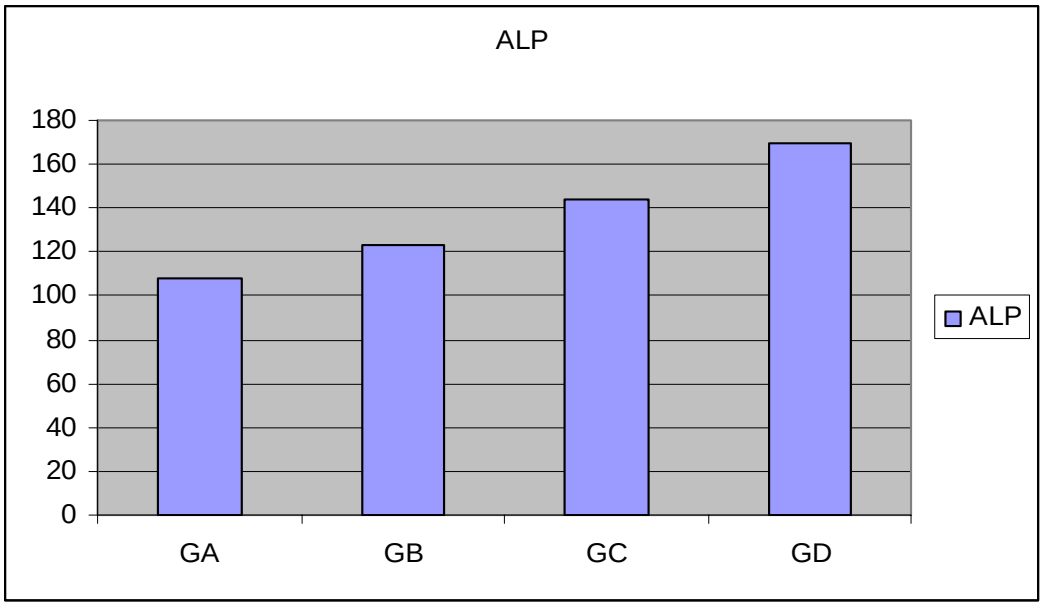

Fig [12]: Changes in ALP activity in rats treated with various levels of MSG.

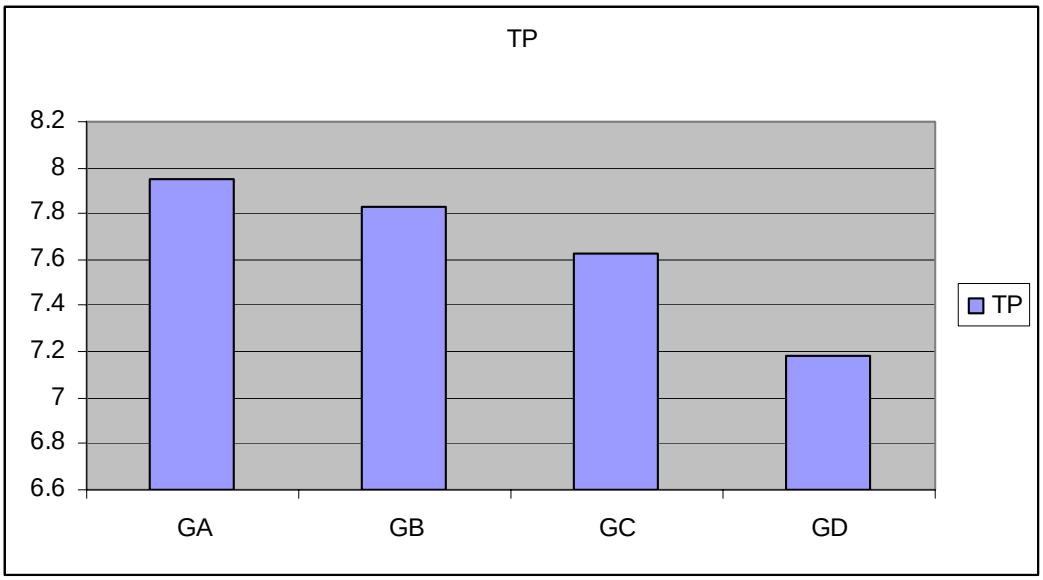

Fig [13]: Changes in total protein concentration in rats treated with various levels of MSG.

3. 5 Histopathological Finding

In group (B) which treated with 120 mg/kg Bwt the brain showed vaculization and infiltration of gilial cells [fig14]. The liver showed congestion of central veins and hydropic degeneration of hepatocytes characterized by microvesiles inside the cytoplasm [fig 15].

In group (C) which treated with 240 mg/kg Bwt the brain showed vaculization with congestion of blood vessels and pycnotic neucli [fig 16]. There was congestion of central vein and sinasoides with pycnotic neucli and small microvacules in cytoplasm in the liver within the same group [fig 17].

In group (D) which treated with 480 mg/kg Bwt the brain was congested and showing heamorrhage and infiltration of inflammatory cells in the meninges [fig 18]. Congestion of central veins and portal areas were seen in liver [fig 19].

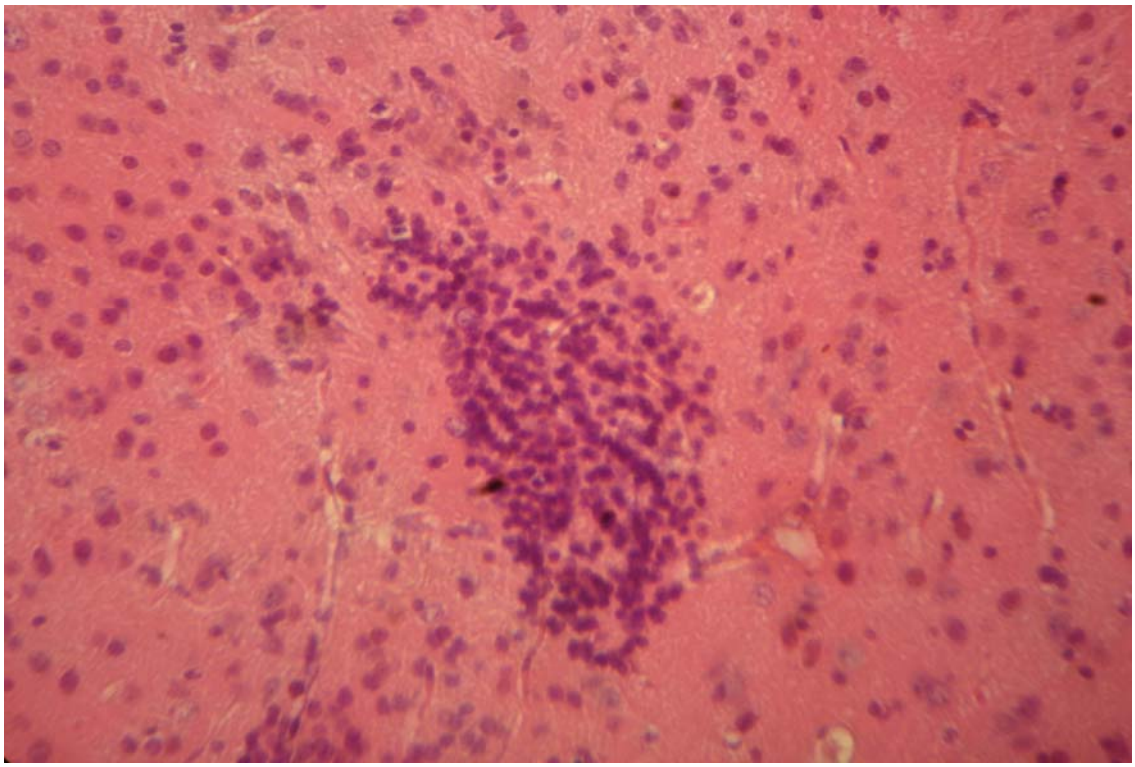

Fig [14]: Brain section from rat received 120 mg/kg body weight notice vaculization and infiltration of gilial cells. (H & E × 40)

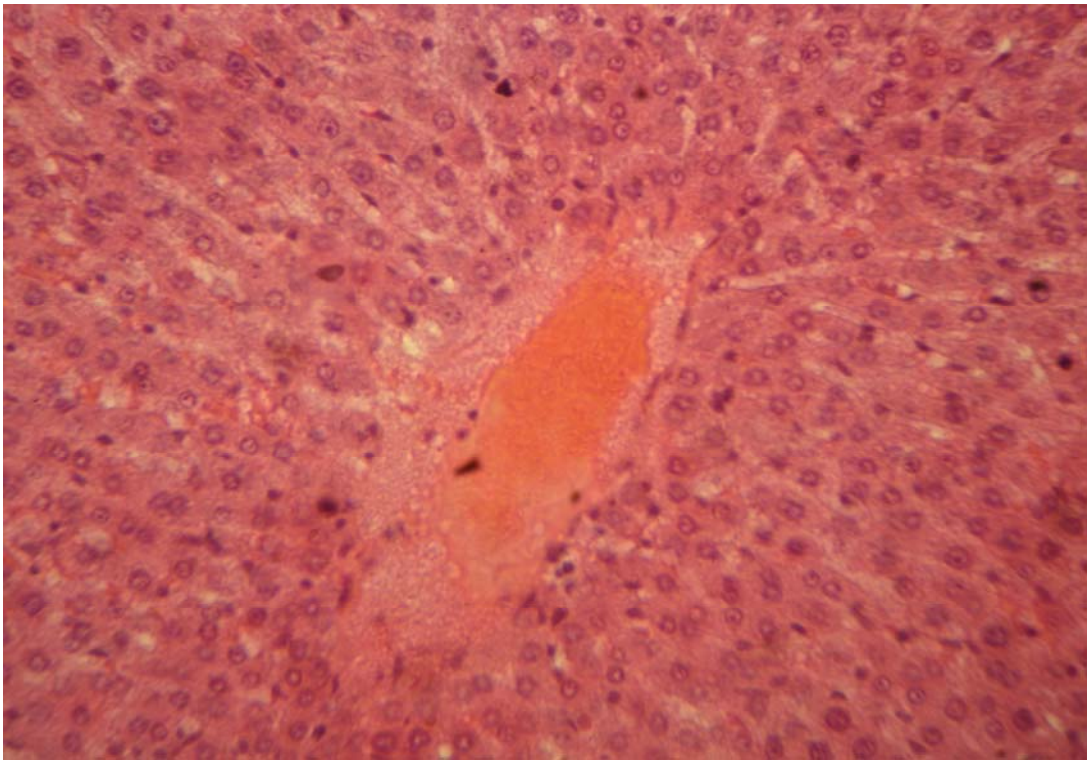

Fig [15]: Liver section from rat received 120 mg/kg body weight notice congestion of central veins and hydropic degeneration of hepatocytes characterized by microvesiles inside the cytoplasm. (H & E × 40)

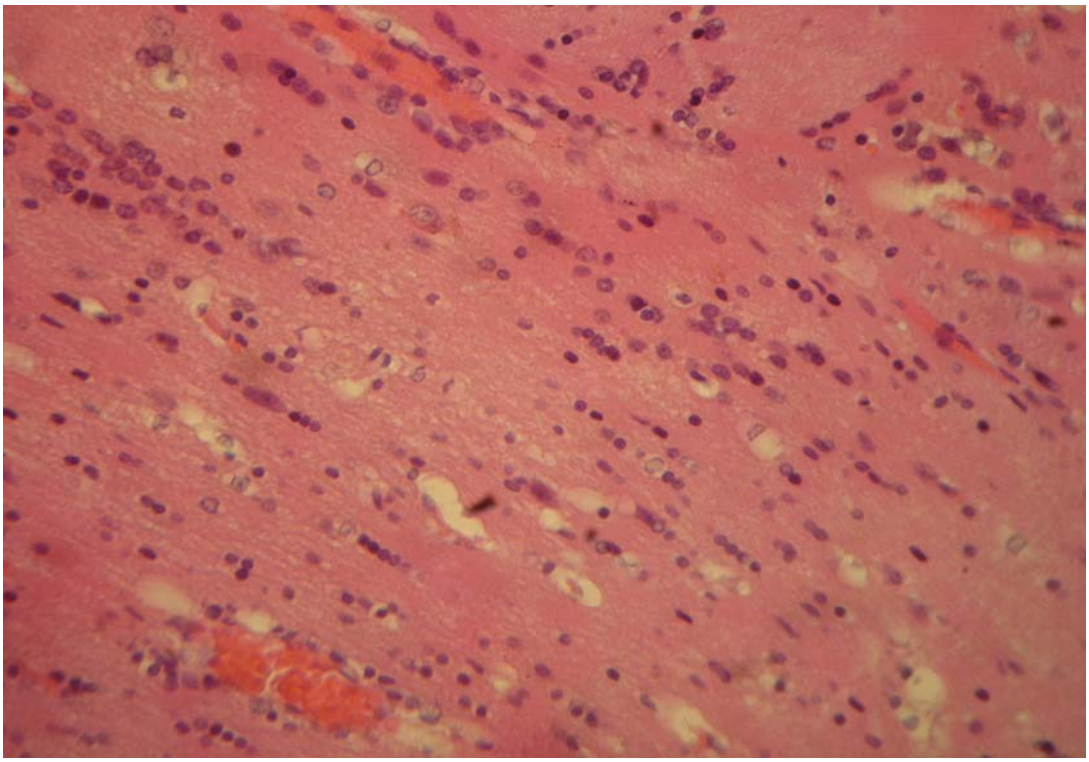

Fig [16]: Brain section from rat received 240 mg/kg body weight notice vaculization with congestion of blood vessels and pycnotic neucli. (H & E × 40)

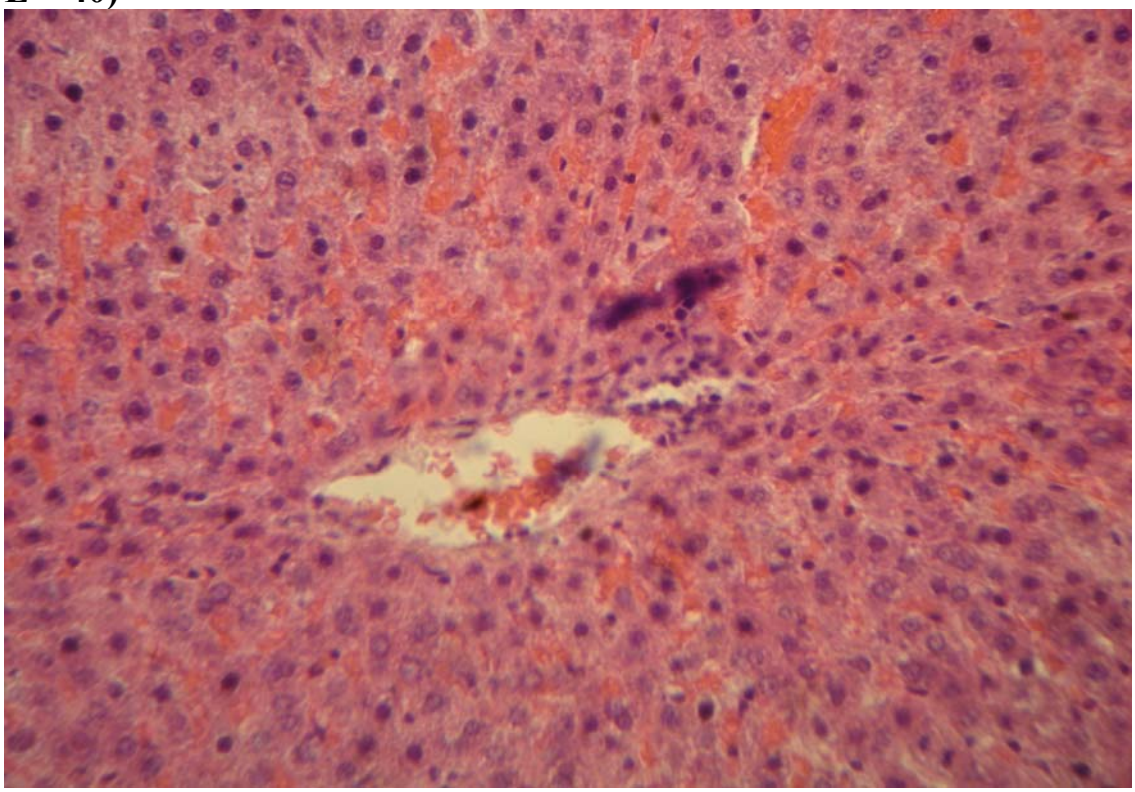

Fig [17]: Liver section from rat received 240 mg/kg body weight notice congestion of central vein and sinasoides with pycnotic neucli and small microvacules in cytoplasm. (H & E ×40)

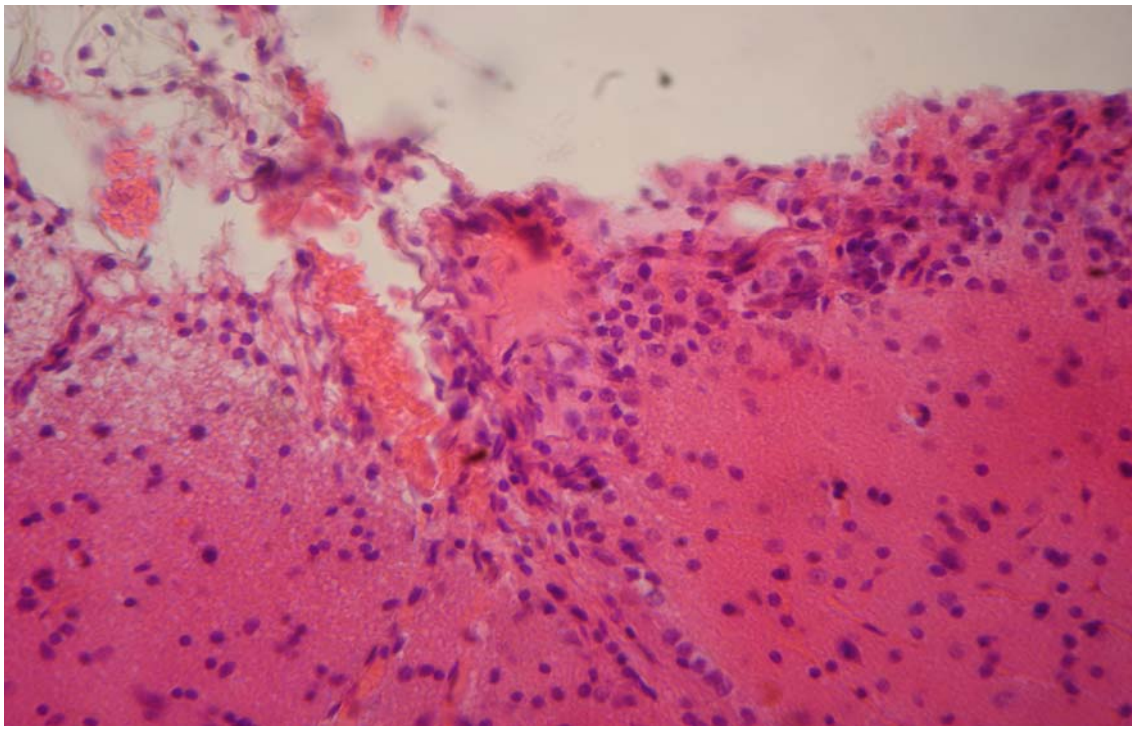

Fig [18]: Brain section from rat received 480 mg/kg body weight notice congestion, heamorrhage and infiltration of inflammatory cells in the meninges. (H & E ×40)

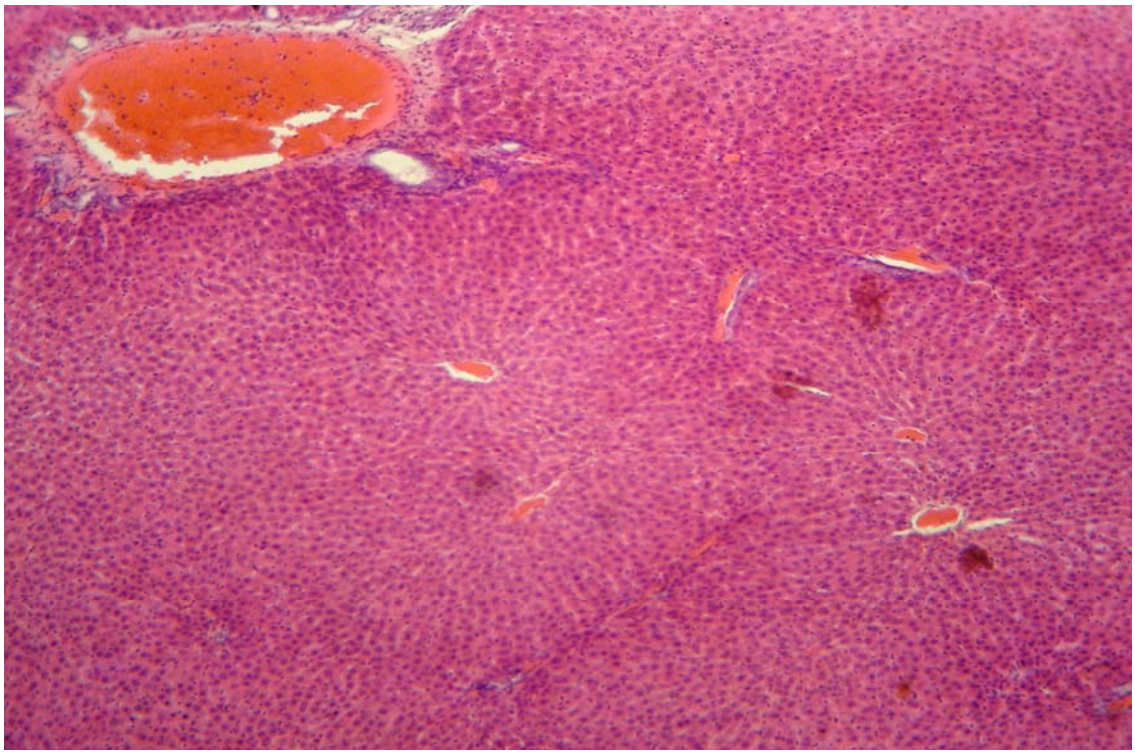

Fig [19]: Liver section from rat received 240 mg/kg body weight notice Congestion of central veins and portal areas. (H & E × 10)

Chapter Four

Discussion

In this study, the groups treated with MSG exhibited clinical symptoms of shiver, dizziness, disorientation, hyperactive with hysterical sings and paralysis of one leg. The severity of symptoms increased with increased levels of MSG.

These results were similar to those reported by Bhagavan *et al.* (1971) which include somnolence and seizures. Tail automutilation reported by Iwata *et al.* (1979).

Similar results were reported by Mohd *et al.* (2000) who claimed that administration of MSG to neonatal rats cause neuronal necrosis of the hypothalamus along with behavioral abnormalities.

Learned taste aversion reported by Vogel and Nathan (1975) and irritability to touch was interpreted as conspicuous emotional changes by Nemeroff *et al.* (1977).

MSG is also known to produce a variety of adverse reactions in some people which include depression, mood swings, rage reactions, migraine headache, dizziness, light-headedness, loss of balance, disorientation, mental confusion, anxiety, panic attacks, hyperactivity, behavioral problems in children, attention deficit disorders, lethargy, numbness or paralysis seizures, shills and shakes, and shuddering (www.truthinlabeling.org).

There were no significant effect on body weight and relative organs weight in the present work in rats treated with MSG. This indicates that MSG has no effect on the growth rate. This is correlated to Hara *et al.* (1962) who reported that natural and synthetic monosodium L-glutamate at different doses when given orally to rats once a day for 90 days had no effects on body-weight and relative organs weight.

Schoelch *et al.* (2002) reported that the postweaning development of MSG obesity and depressed thermogenesis are preceded by an early phase of increased energy expenditure with decreased fat deposition during suckling age and hypothesize cell damage in the arcuate nucleus to be involved in both.

In the present investigation MSG levels have no significant effect on heamatological values and this agreed with the finding of Geha *et al.* (2000) who confirmed that under controlled conditions no objective changes in blood values, except for a transient rise in glutamate levels.

The reduction of total protein in the treated groups in this study may be due to liver damage as illustrated histologically since the liver cells are responsible for protein synthesis. The increase in AST, ALT and ALP activities is an indication of hepatic damage. These are illustrated by pathological changes of the liver. This is in agreement with Cohen (1967) who reported that acute irreversible degeneration in liver and retina of neonatal mice has been seen following parenteral administration of MSG.

Diniz *et al*, (2004) stated that the hepatic glucose metabolic shifting induced by hypercaloric diet intake and MSG administration were associated with oxidative stress in hepatic tissue.

The lesions found in the brain in the present work are indications of brain toxicity. These findings are similar to findings of Olney (1969) who reported that MSG treatment caused brain lesions, particularly acute neuronal necrosis in several regions of the developing brain of neonatal mice, and acute lesions in the brains of adult mice given 5 to 7 mg/g of MSG subcutaneously. Lemkey-Johnston and Reynolds (1974) also confirmed that MSG induces neurotoxicity.

Pardrige (1979) illustrated that dietary glutamate does not enter the brain because the blood-brain barrier maintains the transport system for the

acidic amino acids, such as glutamate, to effectively exclude circulatory glutamate from the brain.

Several areas in the brain are normally do not have a barrier system, the circumventricular areas, these include the hypothalamus, the subfornical organ, organium vasculosum, area postrema, pineal gland, and the subcommisural organ.

As stated in literature, glutamate is the most important neurotransmitter in the hypothalamus (Shank and Aprison, 1988), Therefore careful regulation of blood levels of glutamate is very important, since high blood concentrations of glutamate would be expected to increase hypothalamic levels as well.

Exposure to MSG caused damage to arcuate of the hypothalamus as indicated by Olney *et al*, (1977). Takasaki (1979) stated that MSG induces hypothalamic damage when given to immature animals after either subcutaneous or oral doses.

Chronic elevations of blood glutamate can even seep through the normal blood-brain barrier when these high concentrations are maintained over a long period of time (Toth and Lajtha, 1981).

Coyle *et al*, (1981) indicated that the damage by monosodium glutamate was much more widespread, including the hippocampus, circumventricular areas, locus cereulus, amygdala- limbic system, subthalamus, and striatum**.**

Sasaki *et al*, (1994) reported similar results of cell damage in the arcuate nucleus. This is one of the most distinct neurotoxic effects of early postnatal MSG application, and occurs within a few days.

Moreover, the blood brain barrier is easily damaged by fever, stroke, and trauma to the head, seizures, ingestion of processed free glutamic acid, and the normal process of aging (Blaylock, 1994).

Free glutamic acid including processed free glutamic acid can cross the blood brain barrier in an unregulated manner during development, and can

pass through the five circumventricular areas, which are leaky at best at any stage of life (Skultaetyovaa *et al*, 1998).

Park *et al*, (2000) found that single intraperitoneal injection of 4.0 mg/g bodyweight of MSG caused significant damage to hypothalamic neurons in the arcuate nucleus and impaired memory retention in adult mice. Gonzalez-Burgos *et al*, (2001) found that subcutaneous administration of 4.0 mg/g bodyweight of MSG to male neonate rats induced excitotoxicity, leading to cell death in prefrontal cerebral cortex.

Martinez-Contreras *et al*, (2002) reported administration of 4.0 mg/g body weight of MSG caused reactivity of astrocytes and glial cells in the frontoparietal cortex, including hyperplasia and hypertrophy.

In 1968, the first of the observable adverse human reactions to glutamate following ingestion of glutamate in food form were noted (Kowk, 1968).

Since that time, the list of observable adverse reactions has been growing.

To date little or no research has focused on the mechanisms which cause the observable adverse reactions to glutamate; although Zorumski (1990) has suggested that research focusing on exogenous food excitotoxins serves as a promising source of information for brain research.

Conclusions and Recommendations

From the results obtained, we can conclude that:

(1) MSG cause clinical symptoms which may be related to lesions in the brain and the liver.

(2) MSG has no effect on the rate of growth.

Further studies can be done on the effect of MSG for longer period of time.

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