
EFFECTS OF ADMINISTRATION OF MONOSODIUM L-GLUTAMATE ON THE SERUM ACTIVITIES OF SOME LIVER ENZYMES, SERUM TOTAL PROTEIN AND LIVER HISTOMORPHOLOGY OF WEST AFRICAN DWARF GOATS

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ABSTRACT

This study investigated the effects of monosodium L-glutamate (MSG) administered orally or subcutaneously to male West African Dwarf (WAD) goats on the serum activities of some liver enzymes, serum total protein and liver histomorphology. Twenty-eight adult male WAD goats were used for the study. They were randomly assigned to 7 groups (A, B⁰, B^S, C⁰, C^S, D⁰ and D^S) of 4 goats each. Group A served as untreated control; B⁰ and B^S received 0.25 g of MSG/kg body weight (bw) orally or subcutaneously (S/C), respectively; C⁰ and C^S received 0.5 g of MSG/kg bw orally or S/C, respectively; and D⁰ and D^S received 1 g of MSG/kg bw orally or S/C, respectively. The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and serum levels of total protein were assayed on days 0, 14 and 28 of MSG administration, and liver sections obtained at day 28 were processed for histomorphological evaluation. The results showed that on days 14 and 28 the serum ALT and AST activities of goats in the treated groups were significantly lower ($p < 0.05$) than those of the untreated control group. The serum total protein of all the treated groups were significantly higher ($p < 0.05$) than that of the untreated control group on day 14, but on day 28, there was no significant variations ($p > 0.05$) in the serum total protein of the treated and untreated groups. No obvious histomorphological lesions were observed on the liver tissues on histomorphological examination. It was concluded that MSG administration as used in this study in WAD goats led to significant reductions in serum ALT and AST activities and elevation of serum total protein, which is suggestive of enhanced hepatocyte membrane stabilization and hepatic protein synthetic ability.

Keywords: L-glutamate, Serum, Alanine, Aspartate, Transferase, Proteins, Liver, Goats.

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INTRODUCTION

Monosodium L-glutamate (MSG) is the sodium salt of the non-essential amino acid glutamic acid – one of the most abundant amino acids found in nature. Interest in the toxicity of MSG as a food additive has increased greatly in the past years because of the association of this compound with both Chinese restaurant syndrome in humans and lesions of the hypothalamus in new-born mice and monkeys [1]. Adverse reactions to food can be defined as any abnormal physiological response to a particular food [2] and can be classed into a number of different categories, with two major categories being toxic reactions and hypersensitivity reactions [3]. Toxic reactions will occur in virtually all individuals in a dose-dependent manner, whereas hypersensitivity reactions are usually idiosyncratic reactions that only occur in a small subset of individuals [4]. Sensitivity to most food additives is believed to occur in only a small minority of the population [5, 6], with most adverse effects being attributed to various pharmacological and other non-immunological mechanisms [7], rather than being true allergic reactions.

The liver is an organ of diverse metabolic activities involving synthesis, biotransformation and storage of numerous substances. Any assessment of its functional status is usually based on its ability to perform specific metabolic functions. Hepatocellular integrity and the functional status of the liver and such other notable organs like kidneys and pancreas can be evaluated by laboratory tests [8, 9]. The clinical manifestations of diseases of these organs are not usually outwardly characteristic. This very reason necessitates the application of certain laboratory tests to detect the presence of such disorders. A good number of tests have therefore been developed for the detection of alterations in hepatocellular integrity and liver function, with only a few having been found to be practicable and useful in veterinary practice [10].

The effects of MSG have been studied in many animal species [11], but available literature show little or no such studies in West African dwarf WAD goats. More so, no reported studies have been found that compared different routes (oral and subcutaneous) of administration of MSG either within or between animal species [12]. The WAD goat is an economically important breed of goat commonly raised under semi-intensive or extensive husbandry methods in Africa. These animals mostly roam about scavenging for food. Through scavenging, they have access to leftover foods and leaves used in wrapping such foods cooked with MSG. Even the owners of such goats that are strictly reared intensively feed their goats with leftovers and supplements that may contain MSG. More importantly, the *Fulani* animal rearers in Nigeria traditionally use MSG in “knocking out” libido in bucks [13]. Though this is a common practice, there is little or no information in available literature on the effects of administration of MSG on hepatocellular integrity and synthetic ability of the liver of the goats. The objective of this study was to evaluate the effects of oral or subcutaneous administration of varied doses of MSG on the serum activities of some liver enzymes, serum total protein and liver histomorphology of male WAD goats.

MATERIALS AND METHODS

Experimental Animals

Twenty-eight adult male WAD goats were used for the study. They were 12 to 15 months of age, and weighed between 8 and 12 kg. The goats were acclimatized for two weeks before commencement of the study. During the acclimatization period, they were vaccinated against *peste des petites ruminants* (PPR) using the PPR vaccine obtained from National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. They were also dewormed with mebendazole (Wormin®) manufactured by Cadila Pharmaceuticals, Dholka, India [14]. After the two weeks period of acclimatization, they were randomly assigned into groups and tagged. They were housed within the Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed *ad libitum* on common edible grasses and shrubs (*Panicum maximum*, *Stylosanthes gracilis* and *Penisetum purpureum*) in addition to feed supplements. Drinking water was also provided freely.

Monosodium L-Glutamate

The test compound used was Vedan[®] (99% MSG) brand of monosodium L-glutamate (MSG) (C₅H₈NNaO₄·H₂O), manufactured by Vedan Enterprise Corporation, Taiwan. It was dissolved in distilled water before use [15]. A stock solution was prepared by dissolving a 454 g sachet of MSG crystals in distilled water and made up to 1620ml volume.

Experimental Design

The 28 male WAD goats were randomly assigned into seven groups (A, B^O, B^S, C^O, C^S, D^O and D^S) of 4 each. Group A served as untreated control and therefore did not receive MSG. Groups B^O and B^S received 0.25 g of MSG/kg body weight (bw) orally and subcutaneously (O/SC), respectively. Groups C^O and C^S received 0.5 g of MSG/kg bw O/SC, respectively. Groups D^O and D^S received 1 g of MSG/kg bw O/SC, respectively. These doses were chosen to simulate the reported daily intakes of humans which we believe is directly related to the quantity that goats may possibly be exposed to [16,17,19]. Moreover, MSG has been shown to have very low acute toxicity with LD50 of 15 mg/kg and 18 mg/kg in rats and mice respectively [19]. The MSG administration (MSGa) was 48 hourly for a period of four weeks. The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and serum levels of total protein were assayed on days 0, 14 and 28 of MSG administration. The goats were humanely sacrificed on day 28 of the experiment and samples of their liver tissues were collected for histomorphological evaluation.

Evaluation of Serum Activities of ALT and AST

The serum activities of ALT and AST of the male WAD goats treated with MSG together with the controls were determined on days 0, 14 and 28 of MSG administrations following the Reitman and Frankel colorimetric method [20] for *in vitro* determination of ALT and AST in serum or plasma, using a Quimica Clinica Aplicada (QCA) test kit (Quimica Clinica Aplicada, Spain).

Evaluation of Serum Levels of Total Protein

The serum total protein levels of the goats treated with MSG together with the controls were determined on days 0, 14 and 28 of MSG administrations following the direct Biuret method [21] for the *in vitro* determination of total protein in serum or plasma, using Quimica Clinica Aplicada (QCA) total protein test kit (QCA, S. A. Spain).

Histomorphological Evaluation of the Liver

The liver tissues were fixed by immersion in Bouin's fluid for 48 hours. Later, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. Five micrometre thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd. 1999-2004).

Data Analysis

Data generated were subjected to one-way analysis of variance (ANOVA). Variant means were separated using least significant difference (LSD) method. Significance was accepted at probability level less than 0.05.

Ethics

The housing, handling and welfare of the goats used for the study followed the standards in accordance with the Ethics and Regulations guiding the use of research animals in the University of Nigeria, Nsukka.

RESULTS

There were no significant variations ($p > 0.05$) in the serum ALT activity of all the goat groups before commencement of MSG administration, but on days 14 and 28 of MSG administration, the serum ALT

activity of all the treated groups were significantly lower ($p < 0.05$) than those of the control group that was not given MSG (Table 1). However, within the treated groups, there were no significant variations ($p > 0.05$) between those given MSG by oral or subcutaneous route and the varied doses (Table 1).

There were also no significant variations ($p > 0.05$) in the serum AST activity of all the goat groups before commencement of MSG administration (Day 0), but on day 14 of MSG administration, the serum AST activity of all the treated groups were significantly lower ($p < 0.05$) than that of the control group except those of groups C^O (0.5g/kg MSG orally) and D^S (1.0g/kg MSG subcutaneously) (Table 2). On day 28 of MSG administration, however, the serum AST activity of all the treated groups (without any exception) were significantly lower ($p < 0.05$) than that of the untreated control group (Table 2).

The serum total protein of all the goats groups did not show any significant variations ($p < 0.05$) before the commencement of MSG administration, but on day 14 of MSG administration, the serum total protein of all the treated groups (except group B^S treated with 0.25g/kg MSG subcutaneously) were significantly higher ($p < 0.05$) than that of the untreated control group (Table 3). On day 28 of MSG administration however, there were no significant differences ($p > 0.05$) between the serum total protein of the treated and untreated control groups (Table 3). Microscopic examination of sections of the liver of the goats given varied doses of MSG orally and subcutaneously for 28 days showed no obvious lesions or histomorphological abnormalities.

Table 1: Serum alanine aminotransferase (ALT) activities of male West African Dwarf (WAD) goats given varied doses of monosodium L-glutamate (MSG) orally and subcutaneously.

Groups, with treatments and route where applicable	Means, with standard error of mean in brackets (IU/L)		
	Day 0	Day 14	Day 28
Group A (Untreated Control)	26.09 (3.07)	28.25 ^a (3.62)	26.85 ^a (2.44)
Group B ^O (0.25g/kg bw MSG, oral)	21.04 (1.54)	10.94 ^b (2.85)	9.95 ^b (1.15)
Group B ^S (0.25g/kg bw MSG, subcutaneous)	23.98 (4.20)	9.93 ^b (1.01)	7.96 ^b (0.99)
Group C ^O (0.5g/kg bw MSG, oral)	24.41 (4.01)	10.94 ^b (1.64)	9.95 ^b (1.92)
Group C ^S (0.5g/kg bw MSG, subcutaneous)	24.405 (3.149)	8.928 ^b (2.013)	8.538 ^b (1.433)
Group D ^O (1.0g/kg bw MSG, oral)	27.35 (8.45)	13.96 ^b (3.44)	6.66 ^b (1.57)
Group D ^S (1.0g/kg bw MSG, subcutaneous)	20.62 (1.26)	10.94 ^b (4.03)	7.66 ^b (1.67)

^{ab} Different superscripts in a column indicate significant difference between the means ($p < 0.05$).

Table 2: Serum aspartate aminotransferase (AST) activities of male West African Dwarf (WAD) goats given varied doses of monosodium L-glutamate (MSG) orally and subcutaneously.

Groups, with treatments and route where applicable	Means, with standard error of mean in brackets (IU/L)		
	Day 0	Day 14	Day 28
Group A (Untreated Control)	83.74 (8.05)	89.90 ^a (6.90)	84.24 ^a (8.57)
Group B ^O (0.25g/kg bw MSG, oral)	81.67 (8.50)	66.79 ^b (2.22)	49.05 ^b (3.12)
Group B ^S (0.25g/kg bw MSG, subcutaneous)	83.09 (7.02)	65.45 ^b (6.86)	50.94 ^b (9.18)
Group C ^O (0.5g/kg bw MSG, oral)	86.25 (9.45)	74.58 ^{ab} (4.90)	55.64 ^b (4.92)
Group C ^S (0.5g/kg bw MSG, subcutaneous)	80.45 (2.14)	67.44 ^b (5.61)	53.99 ^b (3.97)
Group D ^O (1.0g/kg bw MSG, oral)	86.14 (9.41)	71.98 ^b (5.84)	58.87 ^b (8.59)
Group D ^S (1.0g/kg bw MSG, subcutaneous)	81.21 (5.57)	73.92 ^{ab} (5.77)	54.61 ^b (6.98)

^{ab} Different superscripts in a column indicate significant difference between the means ($p < 0.05$).

DISCUSSION

The results of the ALT and AST determination suggest that MSG at the doses administered to the WAD goats exhibited a hepatocyte membrane stabilizing effect [22,23]. It is worthy of note that this supposed membrane stabilizing effect was neither dose-dependent nor route-dependent. It is also possible that at the dose levels used in this study, the administered MSG may have caused a reduction of serum ALT and AST by causing an alteration in pyridoxal phosphate – a cofactor necessary for the action of the aminotransferases [24,25,26,27]. The reduced serum ALT observed in the treated goats in this study is at variance with 9.15% increase in serum ALT reported by Egbuonu *et al.* [11] after 28 days of administration by oral administration of MSG to male albino rats daily at a dose of 5mg/kg body weight. Two factors could be responsible for the difference. Firstly, this study utilized male WAD goats as against male albino rats which they used. Secondly, the three graded doses (250mg/kg, 500mg/kg and 1000mg/kg) used in this study are quite higher than the lower dose they used. However, their lower dose is by far below what is daily consumed by humans who utilize MSG as food additive [18].

The significant decreases in serum AST and ALT activities recorded in MSG-treated goats also contrast with earlier reports of an elevation that implied hepatotoxicity [28,29,30]. It is believed that the differences between our findings and the contrasting ones cited may be partly due to the differences in animal species used for the studies (rats in cited cases and goats in this present study) and the doses administered. The earlier cited reports used doses ranging from 5-60mg/kg bw while in this present study

it was 250mg/kg to 1,000mg/kg. It thus appears that administration of such high doses in goats has a hepatocyte membrane stabilizing effect or may have altered pyridoxal phosphate metabolism [22,23,24,25,26,27] The absence of any obvious histomorphological abnormalities in the liver sections of the WAD goats that received oral and subcutaneous administration of MSG gives further credence to this observation.

Table 3: Serum total protein levels of male West African Dwarf (WAD) goats given varied doses of monosodium L-glutamate (MSG) orally and subcutaneously.

Groups, with treatments and route where applicable	Means, with standard error of mean in brackets (g/dl)		
	Day 0	Day 14	Day 28
Group A (Untreated Control)	6.30 (0.24)	5.95 ^a (0.12)	6.16 (0.15)
Group B ^O (0.25g/kg bw MSG, oral)	5.97 (0.14)	7.60 ^b (0.72)	7.71 (0.85)
Group B ^S (0.25g/kg bw MSG, subcutaneous)	5.98 (0.17)	6.96 ^{ab} (0.42)	7.20 (0.31)
Group C ^O (0.5g/kg bw MSG, oral)	6.18 (0.19)	7.62 ^b (0.47)	7.95 (0.18)
Group C ^S (0.5g/kg bw MSG, subcutaneous)	6.45 (0.26)	7.75 ^b (0.51)	8.28 (1.43)
Group D ^O (1.0g/kg bw MSG, oral)	6.16 (0.24)	7.43 ^b (0.41)	7.92 (0.45)
Group D ^S (1.0g/kg bw MSG, subcutaneous)	6.24 (0.23)	7.90 ^b (0.49)	8.40 (0.51)

^{ab} Different superscripts in a column indicate significant difference between the means ($p < 0.05$).

The increased serum total protein recorded for the treated groups on day 14 implies that beyond stabilization of the hepatocyte membrane, MSG at the doses administered to the goats enhanced the protein-synthetic activity of the liver. With glutamate as an amino acid, MSG may have supplied the needed substrates which the liver used in synthesizing part of the extra serum proteins recorded for the treated groups. The finding of increased serum total protein in this present study is at variance with the report of Tawfik and Al-Badr [31] that recorded a reduction in serum protein in rats after administration of MSG at the doses of 0.6mg and 1.6mg/g body weight on daily basis for 14 days. It is thought that the differences in the animal species used for the studies (rats in the cited cases and goats in the present study) may principally account for the differences reported, though it is not completely ruled out that an agent could be toxic at a lower dose (as used in the rat studies) and hepatoprotective at a larger dose (as used in this present study).

Based on the results of the study, it was concluded that administration of MSG to West African Dwarf goats at the doses and routes used in this study led to significant reductions in serum ALT and AST, and

elevation of serum total protein indicative of enhanced hepatocyte membrane stabilization and increased hepatic protein-synthetic ability.

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