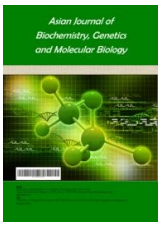

Asian Journal of Biochemistry, Genetics and Molecular Biology

8(3): 14-27, 2021; Article no.AJBGMB.67347
ISSN: 2582-3698

Biochemical effects of Short–long Term Extensive Administration of Monosodium Glutamate and Soybean on Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2021/v8i330194
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal Centre for Advanced Research (MCAR), Muthayammal College of Arts and Science, India.
Reviewers:
(1) Mario Oswaldo Tovar Bohórquez, Universidad del Magdalena, Colombia.
(2) Seyed Pezhman Shirmardi, Nuclear Science and Technology Research Institute (NSTRI), Iran.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67347

Original Research Article

Received 15 February 2021
Accepted 21 April 2021
Published 08 July 2021

ABSTRACT

This study was carried out to investigate the effect of prolonged and excessive consumption of soybean and monosodium glutamate on blood glucose, insulin, and liver function. The quantitative and qualitative determination of oestrogen-like compounds was carried out by chromatography. A total of two hundred and ten (210) Wistar rats (70 – 78g) were divided equally into three groups representing the various experimental durations (2, 4, and 6 months). Each of these groups was further sub-divided equally into fourteen (14) subgroups (7 groups for male rats and 7 groups for female rats). Out of the 7 groups for both the male and female rats, a group represented the control rats only fed commercial rat chow and water, whereas the rest were orally administered any of the 1000 mg/kg b.w (low dose), 2000 mg/kg b.w (medium dose), or 3000 mg/kg b.w (high dose) of aqueous extract of monosodium glutamate or soybean. Diadezein (42.63 mg/100g), and genistein (28.49 mg/100g) were the two most abundant oestrogen-like compounds. After 6 months administration the high dose (H.D) MSG and soybean, significantly altered the blood glucose and insulin levels of both the male and female rats. The liver enzymes levels of the female rats were significantly elevated after 2 months of administration of H.D MSG and soybeans. All the doses of soybean administered for 6 months significantly elevated the liver enzyme levels compared to the

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control. The administration of H.D MSG for 4 and 6 months significantly increased the total bilirubin levels of female rats while no significant changes were observed following soybeans administration. For the male rats, no significant changes were observed on the total bilirubin levels after the administration soybeans, whereas H.D MSG for 2 months significantly increased the total bilirubin levels (12.00 µmol/l) compared to the control (8.60 µmol/l). This study has shown that regardless of the presence of medicinal compounds in soybeans, excessive prolonged intake compromises the functional integrity of the liver while MSG even at minimal doses poses serious health risks.

Keywords: Monosodium glutamate; soybeans; liver enzymes; insulin; glucose.

1. INTRODUCTION

Soybean plays a critical role as an abundant source of dietary protein. The presence of essential amino acids, as well as other macronutrients equivalent to those of rich sources of animals' proteins, makes soybean a complete protein [1]. Soybean is popular among other plant-based proteins due to its high content of isoflavones that provides a gamut of health benefits; cardiomodulation, hepatoprotection, and renal protective effects [2]. Several nutritional intervention preclinical and clinical studies have shown that soybean consumption significantly reduces hepatic triglycerides and cholesterol, low density lipoprotein cholesterol (LDL-c), and serum total cholesterol [3]. The few studies that have assessed the hepatic effects of soybean suggest that altered liver transaminase activities are restored upon extended consumption [4], hence, as these liver transaminases are diagnostic markers for other diseases, soybean consumption could benefit the general wellbeing. Thus, given the widespread consumption of soybean, especially with negative reports regarding the abundance of oestrogenic substances in soybean, the assessment of the biochemical implication of soybean consumption has become critical. Monosodium glutamate (MSG), a sodium salt of glutamic acid, is a food additive that enhances food flavor by inducing a unique taste known as "umami", which has been described as being separate from the basic tastes of sweet, salt, sour and bitter. The umami makes food taste somewhat "brothy", "meaty", or "savory", just similar to the taste of meat. Despite the consideration by food regulatory bodies' like the U.S. Food and Drug Administration (FDA) and National Agency for Food and Drug Administration and Control (NAFDAC) that MSG is Generally Regarded as Safe (GRAS), and its flavor-enhancing potentials [5], its food uses remains contentious at local and international levels. These contentions arose from the anecdotal report that MSG has a hand in series of diseases generally called the Chinese Restaurant Syndrome (CRS). As at that time, palpitations, fever, facial numbness and pressure were common symptoms. Subsequent reports revealed that MSG promotes neurotoxicity, asthma, cardiovascular damage, obesity, and diabetes [6]. There are presently very few studies showing the prolonged and extensive effects of MSG in preclinical settings. Given the widespread use of both MSG and Soybean, the monitoring of these biochemical indices are critical to substantiate which food additive cause or would cause health problems in the longer duration of consumption. On this basis, this study was carried out identify and quantify the constituent oestrogenic substances in soybean, and to evaluate the effects of MSG and Soybean, at three different doses, on the glucose and insulin levels as well as on liver function markers.

2. MATERIALS AND METHODS

2.1 Sample Procurement and Preparation

Ajinomoto a brand of monosodium glutamate (MSG) manufactured by Ajinomoto co., inc. Tokyo, Japan was obtained from Relief Market Owerri Imo State, Nigeria. Soybean used for this study was equally obtained from Ekeonunwa Market Owerri Imo State, Nigeria. Aqueous extracts were obtained on weekly basis for the duration (181 days) of feeding adopted in this study. It was stored and kept away from direct sunlight.

2.2 Analysis of Oestrogenic Substances

The oestrogenous substances extraction was carried out by following the modified method of Liggins [7]. The dried sample was pulverized in a laboratory mortar and pestle. The pulverized samples were weighed and kept for analysis. Precisely, 0.250 g of the sample was dissolved in 20ml of the 80% ethanol and homogenized. It was filtered and washed with 80% ethanol. The

filtrate was evaporated at 45ºC. Five (5) ml of 0.1M acetate buffer, of pH 5.0, was added to hydrolyse the medium. The sample was later extracted three times with 3ml of ethyl acetate. The extract was poured into the round bottom flask of the rotatory evaporator arrangement. It was separated by driving the solvent off the extract. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis. The chromatographic conditions were; Gas Chromatogram HP 6890 synched with software (chemstation 09.01[1206]), injection type was split injection and split ratio was 20:1, the carrier gas was nitrogen, inlet temperature was 250^0C; capillary type AC-5, column dimensions was 30m x 0.25mm x 0.25µm, oven program initial temperature was at 5min for 1hr, 10^0C/min 1st ramping for 20 minutes, 2nd ramping at 15^0C/min for four minutes, detector was flame ionization detector with detector temperature of 320^0C, carrier gas was nitrogen and compressed air pressure 40psi. Regression was used for the verification of concentration response dependence linearity. The peak properties, mainly their retention times aided the identification when compared to the standards. The compounds were quantified from each calibration curves with methanol as internal standard for the phytochemicals.

2.3 Animal Husbandry

A total of two hundred and ten (210) Wistar rats (70 – 78g) were acquired from Dave Animal House, Federal University of Technology, Owerri, Imo State. The rats were acclimatized for 7 days maintained *ad libitum* on water and growers mesh bought from Owerri.

The rats were divided equally into three groups (70 rats each) representing the various experimental durations (2, 4, and 6 months). Each of these groups containing 70 rats were further divided equally into fourteen (14) subgroups, labelled, and orally administered according to the established LD_{50} as shown in the table below:

After completion of the feeding duration, the animals were sacrificed by cervical decapitation under mild anesthesia of ethyl ether. Both blood (collected by cardiac puncture) and sera was prepared for different analysis to be carried out.

The feed intake was calculated from the sum of daily feed intake obtained as the difference between the provided feed and leftover feeds. The weight gain was obtained from the difference in initial and final weights of the rats. The organ weights were obtained from the weights of each eviscerated organs in the electronic weighing balance.

2.4 Biochemical Analysis

2.4.1 Blood sugar test

With a sterile lancet, blood samples were obtained from the tail of the rat. Fasting blood glucose was determined using glucose test strips. The strip was inserted into a glucometer for 5seconds, the process was repeated 3 times for all the animals on and the values recorded.

Table 1. Dosing schedule of rats with MSG and soybeans

Groups	Administration
1	Female rats administered daily 1000 mg/kg b.w (low dose) MSG
2	Female rats administered daily 2000 mg/kg b.w (medium dose) MSG
3	Female rats administered daily with 3000 mg/kg b.w (high dose) MSG
4	Female rats administered daily 1000 mg/kg b.w (low dose) soybean
5	Female rats administered daily 2000 mg/kg b.w (medium dose) soybean
6	Female rats administered daily with 3000 mg/kg b.w (high dose) soybean
7	Female rats fed normal rat chow and water
8	Male rats administered daily 1000 mg/kg b.w (low dose) MSG
9	Male rats administered daily 2000 mg/kg b.w (medium dose) MSG
10	Male rats administered daily with 3000 mg/kg b.w (high dose) MSG
11	Male rats administered daily 1000 mg/kg b.w (low dose) soybean
12	Male rats administered daily 2000 mg/kg b.w (medium dose) soybean
13	Male rats administered daily with 3000 mg/kg b.w (high dose) soybean
14	Male rats fed normal rat chow and water

2.4.2 Estimation of plasma insulin level

The concentration of insulin in serum samples was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method using insulin kit from Syntron Bioresearch (USA). The sample used was non-haemolysed serum. Following a standard procedure, a sample of the standard curve was plotted and insulin concentrations in the samples were determined by interpolation from the standard curve [8].

2.4.3 Liver function parameters

2.4.3.1 Determination of hepatic transaminases

For alanine amino transaminase, fifty microlitres of the sample and five hundred microlitres of the ALT reagent (4-dinitrophenyl hydrazine solution) were mixed in a test tube, and the first absorbance at 340 nm was read after a minute. The timer was started at the same time and further readings of the absorbance were taken after one, two, and three minutes.

ALT activity (nm/min) = 1746 × Δ A 3 40 nm/min, ΔA 340nm/min = change in absorbance per minute for the sample, 1746 = Extinction coefficient.

For aspartate transaminase, fifty microlitres of the sample and five hundred microlitres of the AST reagent were mixed in a test tube, and the initial absorbance at 340 nm was read after a minute. The timer was started at once and further readings of the absorbance were observed after one, two, and three minutes.

AST activity (nm/min) = 1746 × Δ A 3 40 nm/min, ΔA 340nm/min = change in absorbance per minute for the sample, 1746 = Extinction coefficient.

2.4.3.2 Determination of plasma alkaline phosphatase activity (randox method)

Ten microlitre of the sample was dispensed in a cuvette and mixed with five hundred microlitres of the reagent. The initial absorbance was read at 405nm and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation:

ALP activity (IU/l) = 2742 × ΔA 405 nm/min. Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the sample.

2.4.3.3 Determination of total bilirubin concentration (randox method)

Two hundred microlitre of reagent 1 was dispensed into test tube 1 (blank). One drop (Fifty microlitres) of reagent 2 was dispensed into test tube 2 (sample). One thousand microlitres was dispensed into the above two test tubes. Two hundred microlitres of test sample was also dispensed into the two test tubes. They were mixed and incubated for ten minutes at 25°C. One thousand microlitres of reagent 4 was dispensed into the two test tubes. They were mixed and incubated for a further five to thirty minutes at 25°C. The test tubes were inserted in a spectrophotometer and absorbance was read and recorded.
Total Bilirubin (mg/dl) concentration was calculated as 185 x Atb where 185= constant, Atb=Absorbance of Total Bilirubin at (578nm) wavelength.

2.4.3.4 Determination of plasma total protein (randox method)

Three test tubes were assembled, T_1 (blank), T_2 (standard) and T_3 (test sample). T_1 contained 3.0 millilitre-distilled water and 5.0 millilitre biuret reagent, T_2 (standard) contained 3.0 millilitre standard protein solution and 3.0 millilitre biuret reagent. The contents were thoroughly mixed, incubated in a water bath (20 to 25˚C) for thirty minutes; absorbance was read at 560nm against the blank in a spectrophotometer.

2.4.3.5 Determination of plasma albumin (randox method)

Three test tubes were assembled, T_1 (blank), T_2 (standard) and T_3 (test sample). The blank contained 3.0 millilitre bromocresol green reagent and 0.20 millilitre distilled water, the standard contained 5ml bromocresol green reagent and 0.20 millilitre standard protein solution. The test sample contained 5ml millilitre bromocresol green reagent and 0.20 millilitre plasma sample and their contents were mixed and allowed to stand for ten minutes. The absorbance was read against the blank at 630nm in a spectrophotometer

3. RESULTS

3.1 Oestrogen-like Compounds

Table 2 shows the amounts of oestrogen-like compounds found in dehulled soybeans and

soybeans oil. Eleven (11) compounds were isolated from the dehulled soybeans and soybean oil with diadezein (42.63 and 7.96 mg/100g respectively), genistein (28.49 and 2.40 mg/100g respectively), coumestrol (3.88 and 0.23 mg/100g respectively), glycitein (1.98 and 0.28 mg/100g respectively) and diadzein (0.54 and 0.11 mg/100g respectively) being the most abundant oestrogen-like compounds.

3.2. Glucose and Insulin Levels

The glucose and insulin concentration of male and female rats administered varying doses of soybean and monosodium glutamate were represented in Table 3. In the first two months, no significant changes were observed in the glucose and insulin levels of the female rats following the administration of monosodium glutamate and soybeans. After 4 months the administration of high dose of MSG to the female rats significantly elevated the glucose levels (126.75 mg/dl) when compared to the control (97.93 mg/dl) whereas the insulin levels of the female rats (42.96 mg/dl) significantly reduced after 4 months administration of high dose MSG when compared to the control (42.96 ng/ml). After 6 months administration of both the medium dose (M.D) and high dose (H.D) MSG and only H.D soybean, significantly altered the blood glucose and insulin levels of the female rats. For the male rats, the blood glucose and insulin levels remained unchanged after two months administration of soybean and MSG while after 4 months, the high dose of MSG significantly elevated the blood glucose levels (123.61 mg/dl) when compared to the control (102.85 mg/dl) and significantly lowered the insulin levels (52.01 ng/ml) when compared to the control (64.01 ng/ml). The administration of H.D soybeans for 6 months to the male rats significantly elevated the blood glucose levels and decreased the insulin levels.

3.3 Liver Enzyme Concentrations

The hepatic dysfunction markers of rats administered MSG and soybeans were shown in Table 4. The ALT, ALP, and AST levels of the female rats were significantly elevated after 2 months of administration of H.D MSG and soybeans. After 4 months administration of M.D and H.D MSG to the female rats, the ALT levels (120.86 and 123.32 U/L respectively), and ALP levels (311.73 and 320.99 U/L) were significantly elevated when compared to the control. The result showed that 6 months administration of M.D and H.D soybean to the female rats significantly elevated the ALT, ALP, and AST levels, when compared to the control. In the male rats, the low dose administration of MSG and soybeans for 2 months produced no significant changes in the ALT, ALP and AST levels when compared to the control levels whereas the ALT levels were significantly increased by the M.D and H.D MSG while the AST levels was significantly increased by administration of M.D and H.D soybeans. After 4 months administration of M.D and H.D administration of soybean, the ALT levels (139.20 and 135.08 U/L), the ALP (504.50 and 509.50 U/L), and the AST levels (274.00 and 280.50 U/L) were significantly increased when compared to the control levels.

Furthermore, all the doses of soybean administered for 6 months significantly elevated the ALT, ALP, and AST levels when compared to the control levels.

Table 2. Oestrogen-like compounds in dehulled soybeans and soybean oil

Name	Dehulled Soybean (mg/100g)	Oil (mg/100g)
Daidezein	42.63	7.96
Coumestrol	3.88	0.23
Genistein	28.49	2.40
Glycitein	1.98	0.28
Daidzein	0.54	0.11
Genistin	0.36	5.85×10^{-2}
Glycitin	0.26	2.47×10^{-2}
6-o-Acetyldaidzin	1.05×10^{-4}	1.71×10^{-5}
6-o-Acetylgenistin	6.74×10^{-5}	5.70×10^{-6}
6-o-Amalonyldaidzin	1.28×10^{-5}	7.78×10^{-6}
6-o-Malonylgenistin	5.01×10^{-5}	3.85×10^{-7}

Table 3. Glucose (mg/dl) and insulin (ng/ml) levels of rats administered soybeans and MSG

DURATION	GROUPS	Glucose MSG	Glucose Soy	Insulin MSG	Insulin Soy
			FEMALES		
2 MONTHS					
	C	83.7±3.25^{a*}	83.71±3.25^{a*}	59.3±1.41^{a*}	59.31±1.41^{a*}
	L.D	86.3±4.03^{a*}	79.64±9.47^{a**}	53.9±4.24^{a*}	54.08±3.95^{a*}
	M.D	86.5±2.40^{a*}	84.65±6.57^{a*}	54.5±5.79^{a*}	56.35±5.58^{a*}
	H.D	85.4±6.92^{a*}	90.45±2.61^{a*}	56.42±2.68^{a*}	58.75±5.26^{a*}
4 MONTHS					
	C	97.93±1.97^{b*}	97.93±1.97^{b*}	56.54±2.58^{a*}	56.54±2.58^{a*}
	L.D	92.55±2.47^{ab*}	95.75±8.83^{b*}	55.74±2.08^{a*}	53.52±3.23^{a*}
	M.D	95.8±3.11^{ab*}	93.65±3.60^{b*}	51.53±1.38^{a*}	55.35±2.23^{a*}
	H.D	126.75±5.58^{cd*}	103.72±8.20^{b**}	42.96±0.89^{b*}	54.08±1.72^{a**}
6 MONTHS					
	C	97.7±9.33^{b*}	97.71±9.33^{b*}	60.95±4.03^{c*}	60.95±4.03^{b*}
	L.D	90.6±2.96^{ab*}	104.42±10.04^{b*}	56.95±2.33^{a*}	59.65±2.19^{b*}
	M.D	118.7±3.25^{c*}	112.35±5.58^{b*}	47.71±2.26^{b*}	61.65±2.75^{b**}
	H.D	134.55±7.00^{d*}	125.27±6.22^{c**}	39.23±1.36^{d*}	44.65±2.61^{c**}
			MALES		
2 MONTHS					
	C	94.83±6.22^{a*}	94.84±6.22^{a*}	66.42±5.23^{a*}	66.43±5.23^{ac*}
	L.D	94.05±1.76^{a*}	92.65±6.29^{a*}	65.85±4.77^{a*}	70.45±5.72^{a*}
	M.D	91.77±6.50^{a*}	99.05±6.29^{a*}	69.85±2.05^{a*}	72.85±4.17^{a*}
	H.D	98.71±5.79^{a*}	91.15±8.13^{a*}	70.41±6.82^{a*}	75.91±4.06^{a*}
4 MONTHS					
	C	102.85±8.13^{a*}	102.85±8.13^{a*}	64.01±3.23^{a*}	64.01±3.23^{c*}
	L.D	97.30±3.39^{a*}	93.63±6.22^{a*}	62.70±2.24^{a*}	64.32±5.90^{c*}
	M.D	105.44±10.04^{a*}	95.91±7.62^{a*}	65.31±2.56^{a*}	63.50±1.68^{c*}
	H.D	123.61±6.50^{b*}	98.96±1.41^{a**}	52.01±1.96^{b*}	66.12±4.70^{ac**}
6 MONTHS					
	C	93.51±3.81^{a*}	93.51±3.81^{a*}	61.55±3.04^{a*}	61.55±3.04^{c*}
	L.D	102.52±4.80^{a*}	91.55±2.37^{a**}	50.31±1.27^{b*}	65.35±2.47^{c**}
	M.D	123.75±4.73^{b*}	105.45±5.58^{a**}	49.55±1.76^{b*}	54.73±3.11^{d*}
	H.D	142.55±6.15^{c*}	120.95±8.13^{b**}	37.55±2.33^{c*}	45.15±3.88^{e**}

Values are means ± standard deviations of duplicates. Values with different superscript letter(s) (a-e) down the column or symbols (and **) across the row for each parameter, are significantly different (p < 0.05)*

3.4 Total Bilirubin and Conjugated Bilirubin Levels

The result for total bilirubin and conjugated bilirubin levels of the rats administered MSG and soybean were presented in Table 5. The total bilirubin levels of the rats administered the L.D, M.D, and H.D MSG (8.50, 11.50, and 10.50 µmol/l) for 2 months were significantly elevated when compared to the control level (7.00 µmol/l) while administration of L.D, M.D, and H.D soybeans significantly reduced the conjugated bilirubin levels (4.50, 4.00, and 3.00 µmol/l) when compared to the control levels (5.00 µmol/l). The administration of H.D MSG for 4 and 6 months significantly increased the total bilirubin levels while no significant changes were observed in the total bilirubin levels following L.D, M.D, and H.D administration of soybeans but the H.D soybeans and MSG significantly reduced the conjugated bilirubin levels. For the male rats, no significant changes were observed on the total bilirubin levels after the administration of L.D, M.D, and H.D soybeans at all experimental durations, whereas the administration of H.D MSG for 2 months significantly increased the total bilirubin levels (12.00 µmol/l) and decreased the conjugated bilirubin levels (3.00 µmol/l) when compared to the control (8.60 and 6.00 µmol/l respectively). The administration of H.D MSG for 4 months significantly increased the total bilirubin levels of the male rats and significantly reduced the conjugated bilirubin while all the doses of soybean administered to the male rats significantly decreased the conjugated bilirubin levels. All doses of MSG administered for 6

Table 4. Liver function parameters of rats administered monosodium glutamate and soybeans

DURATION	GROUPS	ALT (U/L)	ALT.S (U/L)	ALP.M (U/L)	ALP.S (U/L)	AST.M (U/L)	AST.S (U/L)
		MSG	SOY	MSG	SOY	MSG	SOY
2 MONTHS				FEMALES			
	C	87.00±7.07^{a*}	87.00±7.07^{a*}	261.00±18.38^{a*}	261.00±18.38^{a*}	149.00±29.69^{a*}	149.00±29.69^{a*}
	L.D	82.50±9.19^{a*}	94.00±21.21^{b**}	277.00±31.11^{a*}	353.00±16.97^{b**}	163.00±16.97^{a*}	176.00±24.04^{b**}
	M.D	83.00±4.24^{a*}	95.00±14.14^{b**}	421.50±19.09^{b*}	393.00±14.14^{c**}	153.50±20.50^{a*}	187.50±13.43^{c**}
	H.D	96.00±7.07^{b*}	107.50±13.43^{c*}	456.50±16.26^{be*}	407.50±23.33^{c**}	179.50±14.84^{b*}	184.50±6.36^{c*}
4 MONTHS							
	C	104.86±3.31^{c*}	104.86±3.31^{c*}	293.14±6.63^{c*}	293.14±6.63^{ad*}	176.55±9.12^{b*}	176.55±9.12^{b*}
	L.D	101.46±9.66^{c*}	115.51±5.81^{d**}	288.56±11.22^{c*}	283.63±14.47^{b*}	170.80±10.46^{b*}	180.45±14.07^{bc*}
	M.D	120.86±7.02^{de*}	118.90±5.09^{d*}	311.73±3.72^{d*}	286.65±31.32^{b**}	179.00±13.43^{b*}	208.00±12.44^{d**}
	H.D	123.32±5.40^{d*}	134.06±6.59^{e**}	320.99±10.74^{d*}	301.45±14.49^{d*}	192.00±12.12^{b*}	199.40±14.28^{d*}
6 MONTHS							
	C	116.16±5.17^{e*}	116.16±5.17^{d*}	471.90±12.86^{e*}	471.90±12.86^{c*}	209.40±12.72^{a*}	209.40±12.72^{d*}
	L.D	122.85±9.26^{de*}	161.25±9.97^{fg**}	591.05±17.05^{f*}	599.05±33.58^{d*}	218.75±10.67^{a*}	259.25±9.82^{e**}
	M.D	125.35±7.00^{d*}	155.30±6.78^{f**}	575.00±15.27^{f*}	635.85±20.15^{e**}	266.70±20.08^{c*}	297.70±20.08^{f**}
	H.D	143.55±7.14^{f*}	170.75±4.87^{g**}	580.80±16.26^{f*}	671.70±13.01^{f**}	261.90±17.53^{c*}	305.90±20.50^{f**}
2 MONTHS				MALES			
	C	119.50±12.02^{g*}	119.50±12.02^{hc*}	430.00±18.38^{g*}	430.00±18.38^{g*}	184.50±14.84^{d*}	184.50±14.84^{gc*}
	L.D	118.00±14.14^{g*}	119.00±24.04^{hc*}	425.50±30.40^{g*}	416.00±15.55^{g*}	187.50±9.19^{d*}	215.50±17.67^{hl*}
	M.D	132.50±12.02^{hj*}	128.50±14.84^{h*}	445.00±38.18^{gh*}	501.00±21.21^{h**}	193.00±14.14^{d*}	266.00±9.89^{i**}
	H.D	145.00±22.62^{h*}	143.00±7.07^{i*}	458.50±14.84^{gh*}	512.50±28.99^{hj**}	203.50±19.09^{d*}	288.50±10.60^{j**}
4 MONTHS							
	C	122.03±3.71^{i*}	122.03±3.71^{h*}	463.00±18.38^{h*}	463.00±18.38^{i*}	198.00±11.31^{d*}	198.00±11.31^{kld*}
	L.D	116.81±5.04^{i*}	123.97±4.75^{h*}	453.50±13.43^{h*}	471.50±14.84^{i**}	188.90±12.58^{d*}	207.00±4.24^{ld**}
	M.D	120.95±6.57^{i*}	139.20±5.26^{ie**}	462.50±9.19^{h*}	504.50±12.02^{h**}	208.65±13.64^{d*}	274.00±7.07^{m**}
	H.D	133.80±7.60^{j*}	135.08±6.2^{ie*}	449.50±27.57^{h*}	509.50±23.33^{hj**}	217.90±18.24^{de*}	280.50±12.02^{mj**}
6 MONTHS							
	C	127.70±4.10^{i*}	127.70±4.10^{h*}	523.95±9.40^{i*}	523.95±9.40^{j*}	227.00±5.37^{e*}	227.00±5.37^{h*}
	L.D	126.50±8.90^{i*}	141.40±8.76^{i**}	511.40±25.17^{i*}	657.20±43.84^{k**}	234.35±10.25^{f*}	252.70±9.05^{ne**}
	M.D	140.20±13.29^{h*}	157.30±9.19^{jf**}	552.25±4.31^{j*}	663.05±16.61^{k**}	245.15±7.56^{g*}	260.70±13.85^{nie**}
	H.D	152.00±5.23^{h*}	166.95±10.25^{j**}	582.50±11.73^{j*}	713.30±28.99^{l**}	257.35±16.89^{h*}	295.95±12.23^{of**}

Values are means ± standard deviations of duplicates. Values with different superscript letter(s) (a-n) down the column or symbols (and **) across the row for each parameter, are significantly different (p < 0.05). ALT – Alanine Transaminase, ALP – Alkaline Phosphatase, AST – Aspartate transaminase*

Table 5. Total and conjugated bilirubin levels of rats administered MSG and soybeans

DURATION	GROUPS	TB (µmol/l)	TB (µmol/l)	CB (µmol/l)	CB (µmol/l)
		MSG	SOY	MSG	SOY
2 MONTHS			FEMALES		
	C	7.00±0.00^{a*}	9.00±0.00^{a**}	5.00±0.00^{a*}	5.00±0.00^{a*}
	L.D	8.50±0.70^{b*}	9.50±0.70^{a*}	3.00±0.00^{b*}	4.50±0.30^{b**}
	M.D	11.50±1.94^{c*}	9.00±0.70^{a*}	3.00±1.41^{b*}	4.00±0.00^{b**}
	H.D	10.50±1.66^{cd*}	10.50±3.53^{a*}	3.00±0.00^{b*}	3.00±0.00^{ce*}
4 MONTHS					
	C	10.00±1.41^{d*}	10.00±1.41^{a*}	6.50±0.70^{c*}	6.50±0.70^{d*}
	L.D	9.50±2.12^{d*}	8.50±0.70^{a*}	4.50±0.70^{a*}	5.50±0.70^{a**}
	M.D	9.70±0.70^{d*}	9.50±0.70^{a*}	3.50±0.70^{b*}	4.50±0.70^{a**}
	H.D	15.50±0.70^{e*}	8.00±1.41^{a**}	2.50±0.70^{b*}	3.00±1.41^{bce*}
6 MONTHS					
	C	9.90±2.49^{d*}	9.50±2.12^{a*}	4.50±0.70^{a*}	4.50±0.70^{a*}
	L.D	14.50±0.70^{ei*}	10.20±1.41^{a**}	3.50±0.70^{b*}	4.50±0.70^{a**}
	M.D	15.40±1.01^{e*}	9.00±0.00^{a**}	4.50±0.70^{a*}	5.00±1.41^{a*}
	H.D	17.30±1.39^{f*}	10.50±0.70^{a**}	2.50±0.70^{b*}	3.50±0.70^{bc**}
2 MONTHS			MALES		
	C	8.60±1.41^{gb*}	8.00±1.41^{a*}	6.00±0.00^{d*}	6.00±0.00^{d*}
	L.D	7.50±2.12^{ga*}	9.00±2.82^{a*}	4.00±0.00^{e*}	4.50±0.70^{a*}
	M.D	9.00±1.41^{g*}	9.00±1.41^{a*}	3.00±0.00^{fb*}	4.00±0.00^{a**}
	H.D	12.00±2.82^{h*}	9.50±2.12^{a**}	3.00±0.00^{fb*}	3.50±0.70^{bc**}
4 MONTHS					
	C	8.00±1.21^{g*}	8.00±1.41^{a*}	4.00±0.00^{ea*}	4.00±0.00^{b*}
	L.D	7.00±1.11^{g*}	9.00±1.41^{a**}	3.50±0.70^{fb*}	4.50±0.70^{a**}
	M.D	8.00±1.76^{g*}	9.50±2.12^{a*}	3.50±0.70^{fb*}	4.50±0.70^{a*}
	H.D	13.00±2.82^{h*}	9.40±0.71^{a**}	2.00±0.00^{gb*}	3.00±0.00^{ce*}
6 MONTHS					
	C	7.50±0.70^{g*}	7.50±0.70^{a*}	6.00±1.41^{d*}	6.00±1.41^{d*}
	L.D	13.00±1.41^{h*}	8.00±1.41^{a**}	5.00±0.00^{h*}	5.00±0.00^{a*}
	M.D	14.50±2.12^{hi*}	8.00±1.41^{a**}	4.50±2.12^{eha*}	3.50±0.70^{bc**}
	H.D	13.50±2.12^{hi*}	11.00±1.41^{a*}	3.50±0.70^{fb*}	2.50±0.70^{e**}

Values are means ± standard deviations of duplicates. Values with different superscript letter(s) (a-o) down the column or symbols (and **) across the row for each parameter, are significantly different (p < 0.05). TB – Total Bilirubin, CB – Conjugated Bilirubin*

months on the male rats significantly increased the total bilirubin levels and decreased the conjugated bilirubin levels in comparison to the control, while all the doses of soybean administered for six months significantly reduced the conjugated bilirubin levels.

3.5 Total Protein and Albumin Levels

The total protein and albumin levels of male and female rats administered incremental doses of MSG and soybeans were presented in Table 6. The total protein levels of female rats administered L.D, M.D, and H.D MSG for 2 months (66.50, 62.50, and 55.50 g/l respectively) were significantly lower than the control (74.50 g/l) while the various soybean doses produced no significant changes on the total protein and albumin levels. Administration of H.D MSG for 4 and 6 months significantly decreased the total protein levels (58.25 and 50.75 g/l respectively) and albumin levels (35.26 and 29.77 g/l respectively). All the doses of MSG administered to the male rats for 2 months significantly reduced the total protein levels while only the H.D soybean caused a significant decrease in their total protein levels. No significant changes were observed in the albumin levels of the male rats administered MSG and soybeans for 2 months while 4 months administration of H.D MSG significantly decreased the albumin and total protein levels. Administration of M.D and H.D MSG up to 6 months caused a significant decrease in the total protein and albumin levels while the H.D soybeans significantly decreased the albumin levels.

Table 6. Total protein and albumin levels of rats administered MSG and soybeans

DURATION	GROUPS	TP (g/l)	TP (g/l)	Albumin (g/l)	Albumin (g/l)
		MSG	SOY	MSG	SOY
2 MONTHS			FEMALES		
	C	$74.50\pm6.36^{a*}$	$74.50\pm6.36^{a*}$	$36.35\pm3.13^{a*}$	$36.35\pm3.13^{a*}$
	L.D	$66.50\pm7.77^{b*}$	$73.50\pm6.36^{a**}$	$32.15\pm3.79^{a*}$	$34.71\pm3.92^{a*}$
	M.D	$62.50\pm9.19^{b*}$	$76.50\pm0.70^{a**}$	$30.83\pm2.44^{a*}$	$37.25\pm2.86^{a*}$
	H.D	$55.50\pm6.36^{c*}$	$73.00\pm1.41^{a**}$	$34.26\pm4.01^{a*}$	$35.18\pm2.55^{a*}$
4 MONTHS					
	C	$89.50\pm7.77^{d*}$	$89.50\pm7.77^{b*}$	$45.29\pm2.99^{b*}$	$45.29\pm2.99^{b*}$
	L.D	$69.75\pm11.66^{bf*}$	$91.50\pm3.53^{b**}$	$43.19\pm2.85^{b*}$	$47.21\pm2.09^{b*}$
	M.D	$65.47\pm6.39^{b*}$	$87.50\pm9.19^{b**}$	$40.88\pm2.16^{b*}$	$44.18\pm3.55^{b*}$
	H.D	$58.25\pm8.13^{c*}$	$76.50\pm4.94^{a**}$	$35.26\pm3.42^{a*}$	$46.02\pm2.81^{b*}$
6 MONTHS					
	C	$85.30\pm5.65^{d*}$	$85.30\pm5.65^{b*}$	$47.81\pm3.22^{b*}$	$47.81\pm3.22^{b*}$
	L.D	$67.50\pm2.12^{b*}$	$83.80\pm4.66^{b**}$	$40.50\pm3.87^{b*}$	$44.71\pm3.74^{b*}$
	M.D	$53.80\pm4.66^{c*}$	$84.95\pm3.46^{b**}$	$34.06\pm3.17^{a*}$	$41.61\pm3.50^{b*}$
	H.D	$50.75\pm3.46^{c*}$	$83.15\pm3.60^{b**}$	$29.77\pm3.62^{a*}$	$33.50\pm2.87^{a*}$
2 MONTHS			MALES		
	C	$81.00\pm2.82^{e*}$	$66.00\pm2.82^{c**}$	$39.31\pm3.45^{a*}$	$39.31\pm3.45^{ab*}$
	L.D	$74.50\pm3.53^{fa*}$	$63.00\pm4.24^{c**}$	$32.35\pm2.80^{a*}$	$41.65\pm4.79^{b**}$
	M.D	$75.00\pm5.65^{fa*}$	$68.50\pm2.12^{c**}$	$35.82\pm3.09^{a*}$	$39.02\pm2.61^{ab*}$
	H.D	$78.00\pm8.48^{fa*}$	$73.50\pm7.77^{a*}$	$33.71\pm2.57^{a*}$	$37.63\pm2.90^{a*}$
4 MONTHS					
	C	$74.00\pm5.65^{f*}$	$74.00\pm5.65^{a*}$	$48.36\pm2.43^{b*}$	$47.36\pm2.43^{b*}$
	L.D	$76.50\pm4.94^{f*}$	$75.50\pm2.12^{a*}$	$41.03\pm3.27^{b*}$	$45.90\pm2.83^{b*}$
	M.D	$58.00\pm11.31^{gc*}$	$76.50\pm7.77^{a**}$	$44.69\pm2.05^{b*}$	$42.01\pm3.14^{b*}$
	H.D	$49.70\pm5.23^{g*}$	$69.50\pm7.77^{a**}$	$32.26\pm2.47^{a*}$	$46.23\pm2.16^{b**}$
6 MONTHS					
	C	$90.45\pm4.73^{id*}$	$90.45\pm4.73^{b*}$	$46.42\pm2.92^{b*}$	$46.42\pm2.92^{b*}$
	L.D	$85.35\pm11.66^{id*}$	$86.30\pm2.82^{b*}$	$44.10\pm3.94^{b*}$	$43.94\pm3.07^{b*}$
	M.D	$66.90\pm5.65^{kb*}$	$85.70\pm6.64^{b**}$	$31.29\pm3.64^{a*}$	$45.18\pm2.71^{b**}$
	H.D	$57.70\pm4.94^{lc*}$	$86.50\pm3.67^{b**}$	$29.37\pm2.80^{a*}$	$35.29\pm3.18^{a**}$

Values are means ± standard deviations of duplicates. Values with different superscript letter(s) (a-k) down the column or symbols (and **) across the row for each parameter, are significantly different ($p < 0.05$). TP – Total protein*

4. DISCUSSION

The dietary inclusion of phytoestrogens is currently regulated in many developed countries due to its endocrine disrupting potentials. Exposure to phytoestrogens principally occurs through soybeans and its products. Among the reported phytoestrogens in the dehulled soybeans and its oil, only diadzein, genistein, and coumestrol were found in therapeutic doses. Daidezein and genistein; the two most abundant phytoestrogens in both the dehulled soybean and soybean oil, are isoflavones, while coumestrol is a coumestan. Compared to fruits and nuts [9], vegetables [10], and cereals, the soybean contained therapeutically higher doses of genistein, daidzein, and coumestrol. A study that investigated the biochemical effect of diadzein on Wistar rats found hormone-related influence on energy metabolism and food intake [11]. In humans, recent studies revealed the therapeutic benefits of daidzein in medicine for treating osteoporosis, menopause, cardiometabolic dysfunction, and some hormone-related cancers [11–13]. Genistein is profound for its strong antioxidant properties. It produces atheroproteic effect via the inhibition of the leukocyte-endothelium interaction [14] while its inhibitory effect on hexose transporter, methyl transferases, tyrosine kinases, and topoisomerase has been reported [15–17]. This may provide the scientific backing behind the reported regulatory effect of soybean on cell proliferation and apoptosis [18-19]. The structural formula of coumestrol mimics estradiol enabling the inhibition of 3α-hydroxysteroid

dehydrogenase and aromatase [20], which are enzymes that modulate the biogenesis of steroid hormones [21]. As an estrogen mimic, coumestrol is a potent endocrine disruptor capable of affecting all organ systems that are regulated hormonally via estrogens such as the nervous, skeletal, and reproductive system [22-23]. Also, some studies have associated the suppression of glycogen synthesis and hypocholesterolemic effect of soybean to the coumestrol [24].

Numerous studies have flagged the flagrant consumption of soybeans in various forms [25]. In this study low, medium, and high doses of soybeans were administered to examine the effects on blood glucose and insulin levels. The results showed that both the male and female rats showed adverse biochemical responses in glucose metabolism as the administration of both MSG and soybean persisted beyond 2 months. However, the male rats were more sensitive to incremental doses of the samples than the female rats. This hyperglycaemia found in both male and female rats especially with high doses of soybean might be attributed to potent gastric emptying delays resulting from high viscos arabinogalactans, galactomannans, and pectins that inadvertently delays glucose uptake from the blood stream. This study was in agreement with the reports of Ascencio et al. [26] who observed significant elevations in blood glucose levels on administration of gradient doses of soybeans. On the contrary, Chang et al. [27] found that administration of soybeans longer than 40 days had no effect on blood glucose levels. The insulin levels after administration of low and medium doses of soybeans for 2 and 4 months remained comparable to the control rats. However, all high doses administered for 6 months significantly lowered the insulin levels. In agreement with the findings of this study, Wagner et al. [28] reported that extremely high doses of soybeans could be hypoinsulinemic. In support of this finding, Noriega-López et al. [29] presented a significantly lowered insulin level after excessive intake of soybeans. Hyperinsulinemia has also been shown to be produced in mice due to insulin resistance following soybeans treatment [30]. It is known that insulin plays an important role in increasing glucose intake by cells through inducing the translocation of glucose transporter GLUT4 from intracellular sites to the cell surface. Therefore, hyperglycemia might be attributed to impaired glucose intake by cells due to decreased GLUT4 expression despite hyperinsulinemia. Wagner et al. [28] found that when 8 mg/day of soybean is injected subcutaneously in rats, elevates serum insulin levels and impairs glucose tolerance. This suggests that soybeans may influence insulin release, as the efferent pancreatic branch of vagus nerve could stimulate insulin secretion during the cephalic phase following feeding of additive in rats. MSG has been implicated as a causative agent for several ailments including leptin and insulin resistance [31], possibly influencing energy balance, leading to overweight. Over dose of MSG can increase both insulin secretion and blood glucose level suggesting presence of insulin resistance [32]. In this present study, the effects of gradient doses of MSG administration on glucose and insulin levels were identified. The results indicated that low or medium levels of MSG intake produces minor alterations in blood glucose and insulin levels, however, high dose of MSG significantly elevated the blood glucose levels with a corresponding suppression of insulin secretion. Some reports have shown that intake of L-glutamate causes obesity and diabetic like conditions in rat and mice [33]. This is majorly caused by excessive and prolonged glucose retention in free circulation with decrease insulin levels. Some researchers also proposed that a significant increase in blood glucose after MSG administration was probably because of its conversion glutamic acid moiety of MSG to oxalacetate by D-amino acid oxidase thereby producing glucogenic precursor [6]. Also, in support of the findings of this study, Araujo et al., [34] suggested that the hyperglycaemic effect of MSG could arise as a result of poor ability to oxidize glutamic acid in the pancreatic cells and that MSG acts via phosphoenolpyruvate carboxykinase in decreasing blood glucose. The study of Dayer and Dayer, [35] validates the findings of this present study, showing no significant alterations in plasma insulin concentration in short term administration of low and medium doses of MSG which was attributed to the low stimulation of glutamate receptors in β pancreatic cell [31]. Another theory suggested by Tanizawa et al. [36] was that glutamate alone does not stimulate insulin secretion. The activation of glutamate dehydrogenase enzyme (GDH), stimulates the conversion of glutamate to α- ketoglutarate and play more important role in insulin secretion, thus, GDH stimulation may have remained normal consequently causing no changes in insulin levels. GDH enhances glutamate oxidation and increases ATP production by providing the TCA cycle with substrate (α-ketoglutarate) and therefore

stimulates insulin secretion. Some other studies have also provided conflicting findings on the effect of prolonged MSG intake on insulin levels. Hugues et al. [32] reported a hyperinsulinemic and hypoglycaemic effect of high dose administration of MSG. They found that glutamate lowered blood glucose level during rise in insulin secretion. Macho et al. [37] further explained that insulin resistance in MSG treated group could also be due to changes in insulin binding or post-receptor insulin effects in target tissues.

The evaluation of the hepatic effects of soybeans remains paramount and in this study, low, medium, and high doses of soybean were administered to experimental animals, and their effects on the liver were evaluated. The result showed that changes in concentration of hepatic enzymes mostly occurred with medium and high doses administration from >4 months. Similar findings have been reported by Leng et al. [38] arguing that the soybean when consumed in moderated amounts contains little antinutrients that could cause hepatotoxicity. Furthermore, Wiwanitkit et al. [39] have shown that excessive intake of soybean caused the elevation of liver function markers as well as increase in inflammatory markers from liver homogenates. ALT is regarded as being a more specific indicator of liver inflammation, since AST may be elevated in diseases of other organs such as heart disease or muscle disease [40]. Although ALT and AST are synthesised in the liver, they are also present in serum and in various tissues. In particular, ALT serum levels become elevated during liver diseases, and therefore, it is considered a more specific marker for liver injury than AST [41]. Mild or moderate elevations of ALP or AST are nonspecific and may be caused by a wide range of liver diseases [40]. This means that the prolonged intake of high dose of soybeans could have induced the onset of liver diseases. From the results of this study, the serum levels of ALT and AST were affected by administration of medium and high doses of MSG when compared with the control group. The normal level of ALT in the rats fed MSG is an indication that liver synthetic function was not challenged by low dose administration of MSG. The results of this present study is in agreement with the report of other investigators who have reported no changes of AST and ALT consequent to low and medium dose MSG administration for 28 days [42]. Eweka et al. [43] however observed a gradual increase in AST level after 4 months MSG administration, with a non significant change in ALT. The mean serum activity of alkaline phosphatase was significantly higher in all the groups of rats that received medium and high doses of MSG for > 2months when compared with the control group. This aligns with the study of Zhelyazkov and Stratev, [44]. Alkaline Phosphatase (ALP) is a biomarker enzyme for assessing the integrity of plasma membrane [45]. Increase in the activities of Alkaline phosphatase is an indication that there could be damage due to cytotoxic effect of MSG thereby resulting to leakage of this enzyme from the liver into the serum [44]. Such increase in alkaline phosphatase activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital processes since there may be indiscriminate hydrolysis of phosphate esters in the tissue. The increased ALP activity may also be due to the increased synthesis in the presence of increasing biliary pressure. Again, Rocek et al. [46] demonstrated that MSG administration could alter the intestinal function thereby releasing intestinal ALP.

Both total and conjugated bilirubins are assessments applied to determine both the functional integrity of the liver, and to differentiate between types of liver damage [47]. The elevated total bilirubin levels and consequent decrease in conjugated bilirubin, associated with the MSG administration shows that MSG compromises bilirubin clearance via glucoronic acid conjugation. This is the main predisposing factor to jaundice.

The functional status of the liver is reflected by total protein and albumin levels because the liver is enriched with machineries for the synthesis of serum proteins excluding γ- globulins, hence, liver damage is characterized by hypoproteinemia and hypoalbuminemia which can affect the whole physiological status of animals [48]. In this present study, a significant reduction in total protein and albumin observed in the subjects excessively fed MSG and soybean for 6 months in relation to control group indicates hypoproteinemia and hypoalbuminemia, and by extension a progressive liver damage.

5. CONCLUSION

The study has shown an insignificant disparity across genders for the consumption of monosodium glutamate and soybeans. Furthermore, both the duration and amount of

intake of soybean and MSG is crucial to the both glucose metabolism and the functional integrity of the liver. In general, regardless of the presence of therapeutically active compounds in soybeans, it's excessive and prolonged consumptions alters cellular glucose homeostasis as well as damages the liver, whereas even at minimal doses, MSG still portends a significant health risk.

ETHICAL APPROVAL

Ethical approval has been taken from animal ethics committee to carry out the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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