

Biochemical Effects of Anti-Diabetic Therapies on Obesity and Insulin Resistance Induced by Monosodium Glutamate in Male Rats

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ABSTRACT

Background: This study evaluated the metabolic changes in male obese rats induced by monosodium glutamate (MSG) and determined the possible effect of metformin, Janumet, and Victoza on improving the metabolic changes by assessing the levels of triacylglycerol, cholesterol, fasting blood glucose, glucagon, insulin, HbA1c, leptin, resistin, TNF- α , and interleukin-6. In addition, the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated to assess insulin resistance.

Methods: Rats were orally administered MSG (15 mg/kg of bodyweight [BW]) for 3 months to induce obesity. They were divided into two groups, with MSG continued only in one group. Each group was divided into four sub-groups, namely non-treated MSG obese rats, and those treated with Victoza, Janumet, and metformin, respectively. Bodyweight and other parameters were measured after a month from the beginning of the treatment and data were statistically analyzed.

Results: The MSG obese rats showed a marked increase in the average B.W. and levels of all parameters. Conversely, the lifestyle modification model and all treated groups showed a reduction in average B.W. and a significant improvement in the levels of triacylglycerol, cholesterol, FBS, HbA1c, insulin, HOMA-IR, leptin, resistin, and pro-inflammatory cytokines (TNF- α , and IL-6).

Conclusion: This study indicates that lifestyle modification along with administration of Victoza, Janumet, or metformin improved the carbohydrate and lipid metabolism, insulin sensitivity, and pro-inflammatory cytokines levels. Thus, this treatment strategy could be used for type 2 diabetes mellitus (T2DM) in patients suffering from obesity and insulin resistance.

Keywords: Obesity, MSG, lifestyle modification, IR, pro-inflammatory cytokines

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INTRODUCTION

The number of overweight and obese individuals has doubled since 1980 to an extent that approximately one-third of the global population is overweight. Moreover, obesity rates have increased among both genders and different age groups; it is now considered a public health risk (1). Obesity can harmfully affect physiological functions of the body; it increases the risk of multiple diseases, including insulin resistant T2DM, cardiac disorders (2), cancer (3), musculoskeletal illnesses (4), and reduced mental health (5), which adversely affect the quality of life, work efficiency, and healthcare budgets. Obesity arises from prolonged positive energy equilibrium, i.e., accumulation of fat in the body to an extent that it starts affecting the health (6). A combination of excessive food consumption, lack of physical exercise, and inherited disorders further contributes to it (7). Moreover, the rapid emergence of several food outlets serving delicious but unhealthy, junk food has resulted in the lack of nutrient elements in the body and accumulation of calories (8), which, along with lack of physical movement due to lifestyle changes, has aggravated the situation (9). Furthermore, industrially produced food products have ingredients known as seasonings to adjust deliciousness, increase quality, and extend the lifespan, which exert harmful effects on the health (10, 11). Proper safety checks, based on a series of guidelines, along with determining of end limits, including diagnostic manifestation review, organic chemistry, changes in hematology, and physical examination should be conducted (12, 13). One of the commonly used food flavors is monosodium glutamate (MSG) (E621), a protein-based sodium salt of L-glutamic

acid, which improves the natural aroma of various food products (14). Excessive use of MSG can damage the liver and kidneys. For example, a study reported that administration of MSG to rats resulted in gonadal dysfunction, brain damage, obesity, depletion of several neurotransmitters in the hypothalamus, such as norepinephrine, serotonin, dopamine and their metabolites, stomach cancer, and oxidative stress with progressive alterations in hepatocytes (15). It is speculated that MSG disturbs the energy stability through aggregating diet taste; moreover, it disturbs leptin's hypothalamic signaling pathway (16). Here, we explain that certain food additives, usually considered safe, can result in obesity and related risks. According to a study, losing weight significantly improved the disease condition and lowered the costs of healthcare with a better lifestyle (17). If these measures do not work, pharmacological drugs, which act by lowering the appetite, stomach refilling, or nutrients absorption, could be used to lose weight (18). Although obesity continues to be a major risk factor for increasing insulin resistant T2DM, several conventional therapies for T2DM, for instance, sulfonylureas and insulin, can effectively adjust blood sugar levels and reduce obesity. Such inhibitors include α -glucosidase, amylin mimetics, biguanides, dipeptidyl peptidase-4 (DPP4) inhibitors, GLP-1 agonists, and cotransporter 2 inhibitors of sodium-glucose; these exert either neutral effect on weight or decrease it (19). Metformin (dimethyl biguanide), the first anti-type 2 diabetic (T2D), is widely recommended. Its anti-hyperglycemic effect is primarily attributed to increased insulin signaling, diminished glucagon action, and reduced gluconeogenesis and glycogenolysis (20), with

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the liver as its main target. Furthermore, a study reported unexpected consequences on the effect of metformin on the human gut (21). Pharmacological intervention with a DPP-4 inhibitor, such as sitagliptin (Januvia), clearly provided evidence of its safety and effectiveness. This medication has different modes of action, and patients need to receive it only once a day (22). Sitagliptin prevents the destruction of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) and therefore intensifies insulin secretion. DPP-4 inhibitors have been reported to inhibit the activity of DPP-4 to nearly 100% in vitro and approximately 80% in vivo, thereby extending the half-life of GLP-1 and decreasing HbA1c as well as fasting and postprandial glycemia (23). Liraglutide (Victoza), an equivalent of human GLP-1, has 97% amino acid homology to human endogenous GLP-1, achieved by replacing arginine for lysine at position 34 of endogenous GLP-1. DPP-4 causes rapid deterioration of endogenous GLP-1, with a short-term effect on insulin. Liraglutide consists of fatty acids that bind to albumin and extend its half-life (24). GLP-1 receptors are located in alpha and beta-pancreatic cells, central and peripheral nervous systems, and digestive, lung, and gastrointestinal (GI) tracts. Liraglutide has been reported to induce the secretion of insulin in rats with

high glucose levels by intracellular cAMP, which prevents glucagon secretion and induces gastric emptying (25).

Aim of the work

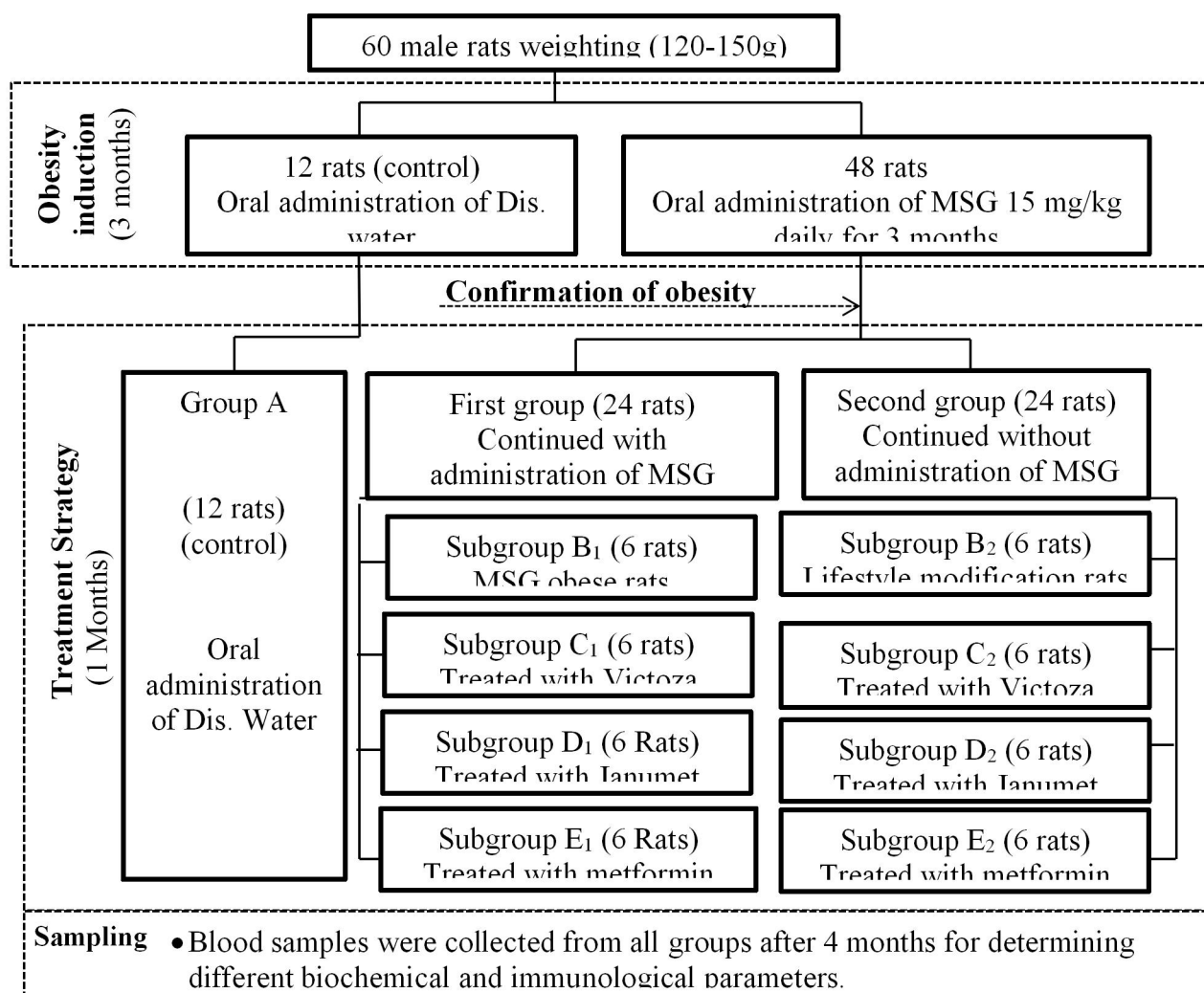
This study evaluated the metabolic changes of MSG-induced obesity in male rats and assessed the possible effects of metformin, Janumet, and Victoza on improving these changes by determining following parameters: triacylglycerol, cholesterol, fasting blood glucose, glucagon, insulin, leptin, resistin, and inflammatory cytokines (tumor necrosis factor- α [TNF- α] and interleukin-6 [IL-6]).

MATERIALS AND METHODS

Sixty male Sprague-Dawley rats, weighing 120 to 150 g, were purchased from the animal laboratory household in the Suez Canal College, Faculty of Veterinary Medicine. They were kept in separate metal animal cages under controlled environmental conditions (20 to 22°C and 55 to 60% relative humidity) and nutritional conditions. They were maintained on a standard balanced diet according to the National Research Center (26).

Bodyweight was measured weekly for adjusting the drug doses (administered according to B.W.). The average B.W. was calculated at the end of the study.

Experimental design



(*) Subgroup B1: MSG obese rats that continued administration of MSG without any treatment.

(**) Subgroup B2: Lifestyle modification continued without administration of MSG, and without any treatment.

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Dose of MSG: (15 mg/kg) orally administrated by gastric tube (27)

Treatment doses

- Victoza dose: (150 µg/kg) subcutaneous injection (28)
- Janumet dose: (50 mg sitagliptin/1000 metformin hydrochloride)/kg b.w. orally administrated by gastric tube (29)
- Metformin hydrochloride: (1000 mg metformin hydrochloride)/kg b.w. orally administrated by gastric tube (30)

The protocol used in the study was approved by the Ethic Committee for animal's use in laboratory experiments of the Faculty of Veterinary Medicine, Suez Canal University, Egypt. Blood samples were collected after 4 months for all groups after overnight fasting from the eye's medial canthus using microhematocrit tubes. Blood samples were divided into two tubes; the first sample (containing EDTA) was used for estimating glycated hemoglobin (HbA1c), whereas the second blood sample was transferred to clean and dry screw-capped centrifuge tubes and centrifuged to separate the serum. Serum samples were used for determining different biochemical parameters, e.g., triglycerides, cholesterol, fasting blood sugar, insulin, glucagon, leptin, and pro-inflammatory cytokines (TNF- α and interleukin-6).

Triacylglycerol levels were estimated using the method described by Siedel et al. (31), whereas total cholesterol was determined using the method described by Allain et al. (32). Fasting blood glucose levels were assessed using the method described by Tietz (33), HbA1c (%) was calculated according to the method described by Zander et al. (34), and fasting blood insulin was determined using the method described by Sapien (35). Glucagon hormone was determined using the method described by Nishino et al. (36). The HOMA-IR was calculated according to the formula for (fasting insulin in the blood (ng/mL) \times fasting blood sugar (mg/dL)/405). Resistin was assessed using the method described by Steppan and Lazar (37), and leptin levels were estimated using the method described by Landt et al. (38). TNF- α levels were determined using the method described by Feldmann and Maini (39), and serum levels of IL-6 (pg/mL) according to the method described by Toga and Kishimoto (40).

Statistical analysis: Data were statistically analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's post hoc multi-range test using Windows SPSS, version 20.0; SPSS Inc., Chicago. The data obtained are expressed as mean \pm standard error (SE).

RESULTS

The average bodyweight, and levels of triacylglycerol and cholesterol increased significantly ($p > 0.001$) in MSG obese rats (subgroup B₁) that received MSG for 4 months compared with the control (subgroup A) (Figs. 1–3). Glutamate affects both directly and indirectly the hypothalamus (41). The subgroup B₁ showed significant increase in fasting blood sugar, HbA1c, and insulin levels when compared with the control. However, no difference in the serum glucagon levels was observed between the subgroup B and control (Figs. 4–7). The subgroup B₁ showed significantly increased HOMA-IR and resistin levels as compared with the control (Figs. 8 and 9). Insulin resistance is characterized by insufficient insulin levels such that the affinity of the receptor is reduced (42). We observed rats in subgroup B₁ showed a marked increase in leptin levels ($p > 0.001$) when compared with control (subgroup A) (Fig. 10). The inability of these high

levels to control bodyweight reflects a mechanism of hormone resistance responsible for disrupting the regulation of bodyweight. Moreover, leptin resistance can impair peripheral physiological processes of leptin such as lipid and carbohydrate metabolism. The subgroup B₁ showed significantly increased levels of TNF- α and IL-6 ($p > 0.001$) when compared with control (Figs. 11 and 12). Lifestyle modified rats (subgroup B₂) and all treated groups showed significantly decreased average b.w. ($p > 0.05$) when compared with the MSG obese rats (subgroup B₁), with no significant difference. However, rats treated with Victoza (subgroup C) showed the best results among treated groups although with significant increase in the average b.w. ($p > 0.05$) when compared with control (Fig. 1). These results are in line with those of Sun et al. (43) who reported that GLP-1 RAs were linked to low b.w. and waist circumference. The lifestyle modified group (subgroup B₂) and all treated groups showed highly significant decrease in triglyceride and total cholesterol levels ($p > 0.001$) when compared with subgroup B₁, with no significant difference (Figs. 2 and 3). These results are in line with that of a study revealing that GLP-1 RAs decreased the triglyceride and total cholesterol levels (43). Treatment with a DPP-4 inhibitor led to two- to three-fold increase in active GLP-1 postprandial (44). Compared to GLP-1 RAs, which can cause an increase of > 10 in GLP-1 (45), we showed that Victoza group demonstrated the best results in reducing the average b.w. Lifestyle modified rats (subgroup B₂) and all treated groups showed a marked reduction in fasting blood sugar levels ($p > 0.05$) when compared with subgroup B₁. However, the subgroup C₂ was the only group that attained normal levels to that of control in FBS. Furthermore, all subgroups in the second group (without MSG) had significantly reduced HbA1c levels ($p > 0.05$) when compared with those that received the same treatment in the first group (with MSG). However, subgroup C₂ showed more improvement in HbA1c levels than other groups (Figs. 4 and 5); these results are in line with those of Buse et al. (46) who reported that Liraglutide increased the regulation of glucose and weight loss. Our findings showed that lifestyle modified rats (subgroup B₂) and all treated groups had significantly decreased insulin levels ($p > 0.001$) when compared with subgroup B₁. However, rats in subgroup C₁ showed the best results among the treated groups, whereas those in the second group without MSG had significantly decreased levels of insulin, without marked differences. There was no notable difference in serum levels of glucagon among subgroup B, subgroup B₂, treated groups, and control (Figs. 6 and 7). This finding showed that subgroup B₂ and all treated groups exhibited reduced HOMA-IR levels ($p > 0.05$) when compared with subgroup B₁. However, subgroup C₂ and subgroup E₂ in the second group (without MSG) showed better results than other groups. Moreover, subgroup B₂ and all treated groups exhibited reduced resistin levels ($p > 0.05$) when compared with group B₁. However, subgroup C showed the best results among the treated groups, whereas there was no significance in the second group (without MSG), except for group D₂ (Figs. 8 and 9). We showed that rats in subgroup C in both groups and those in subgroup E₂ only in the second group (without MSG) showed reduced leptin levels ($p > 0.05$) when compared with MSG obese and lifestyle modified rats (subgroup B) with no

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significant difference in leptin levels when compared with control (Fig. 10).

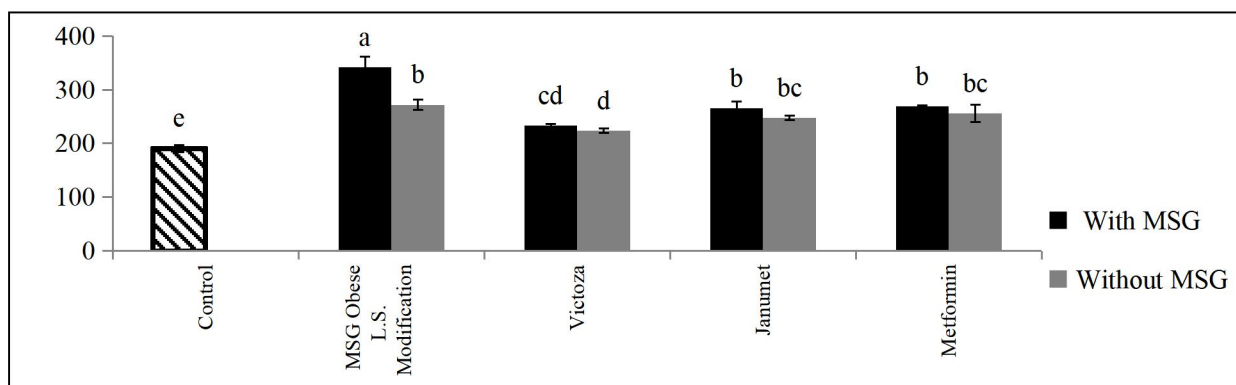
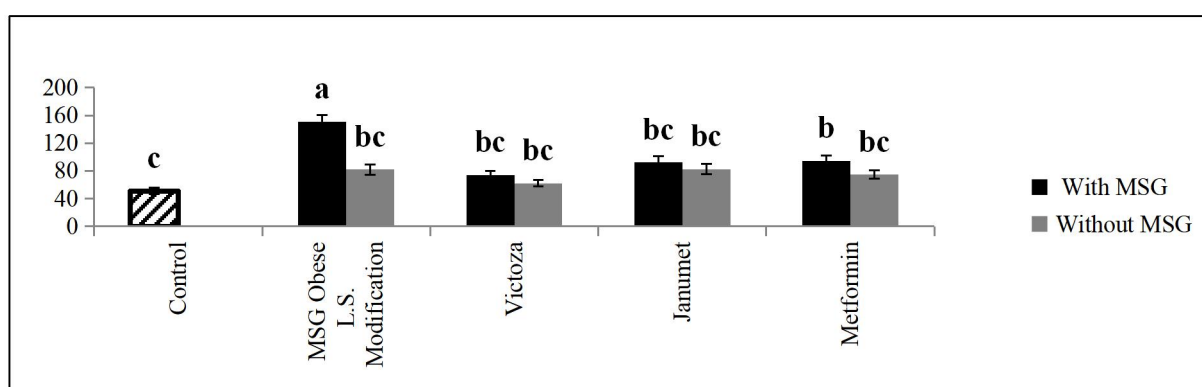


Figure 1 Average bodyweight (g) in different groups

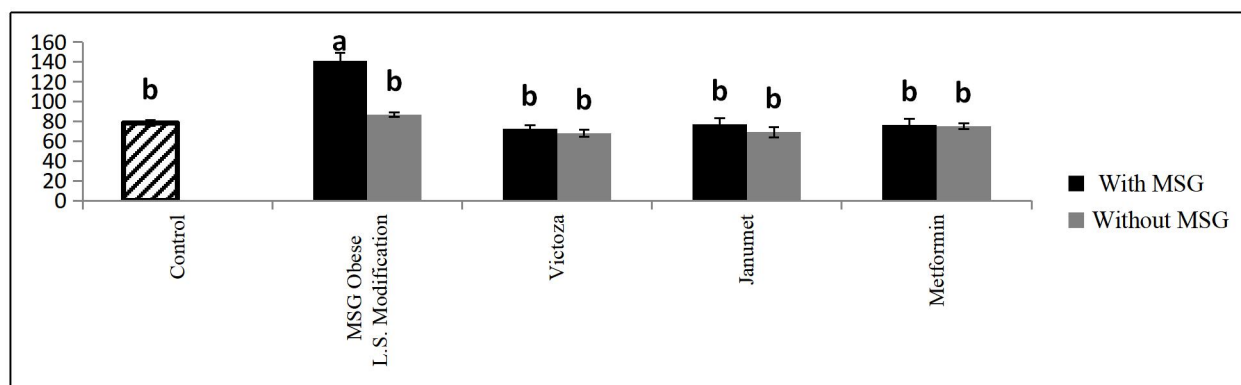
Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 2 Triacylglycerol serum levels (mg/dL) within different groups



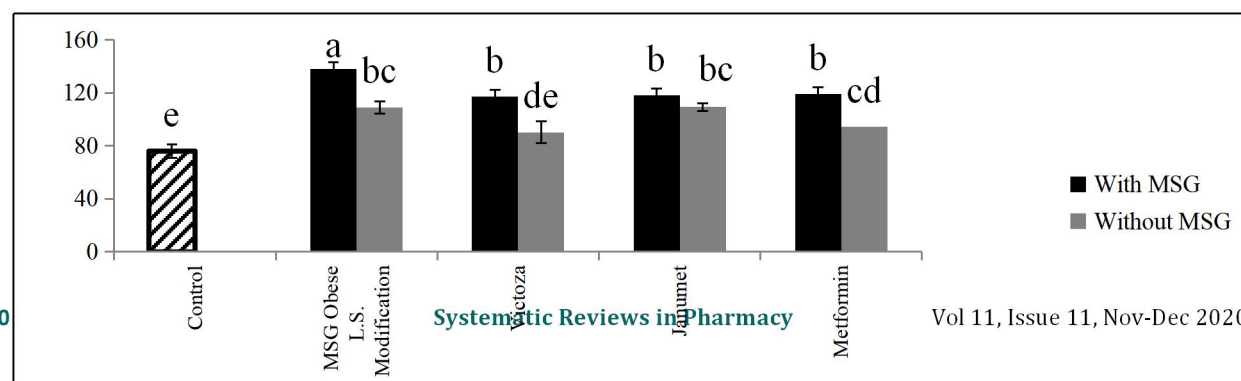
Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 3 Total cholesterol serum levels (mg/dL) within different groups



Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

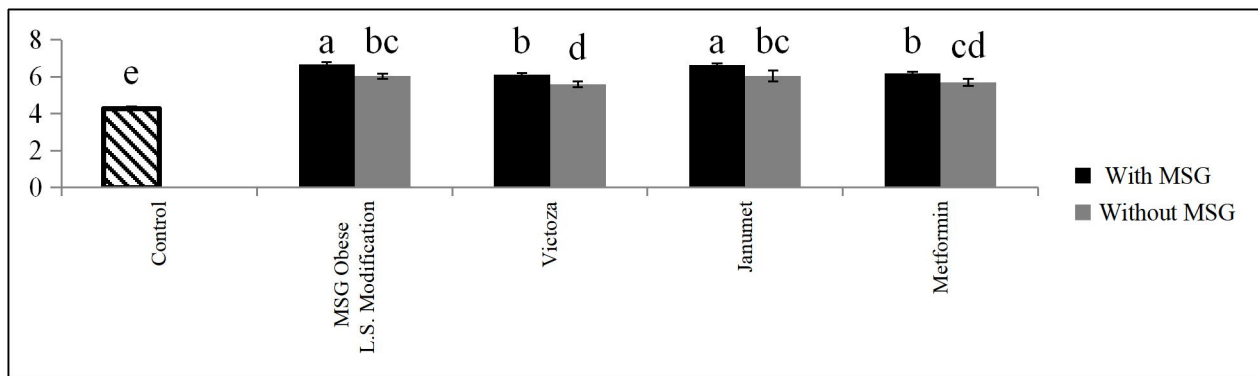
Figure 4 Fasting blood sugar serum levels (mg/dL) within different groups



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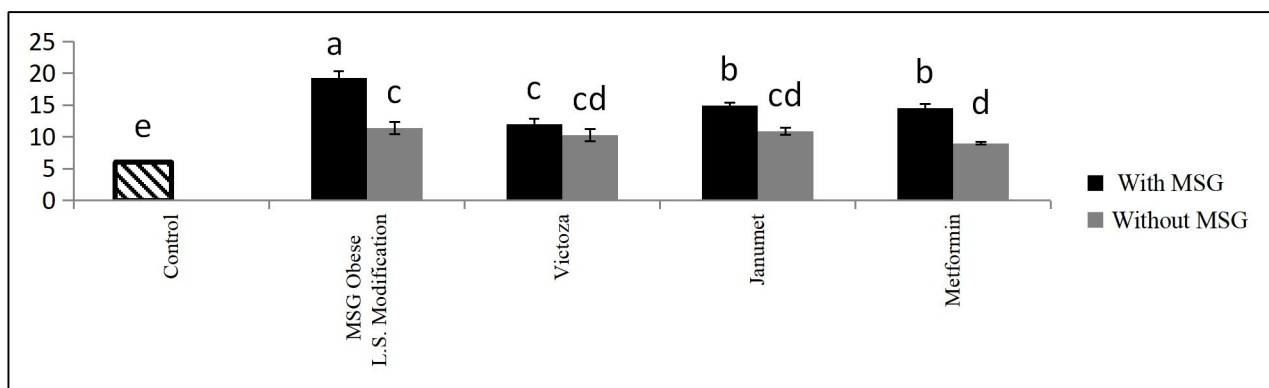
Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 5 HbA1c (%) levels within different groups



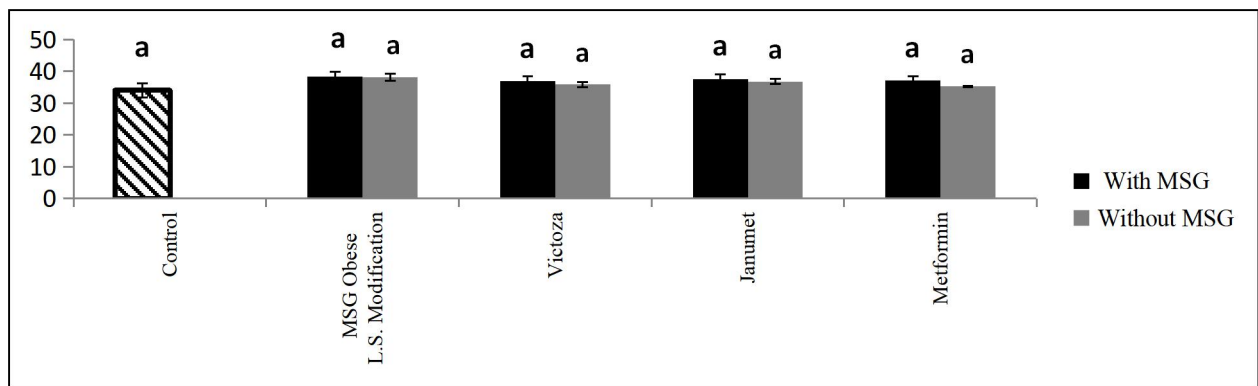
analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 6 Fasting blood insulin serum levels (ng/mL) within different groups



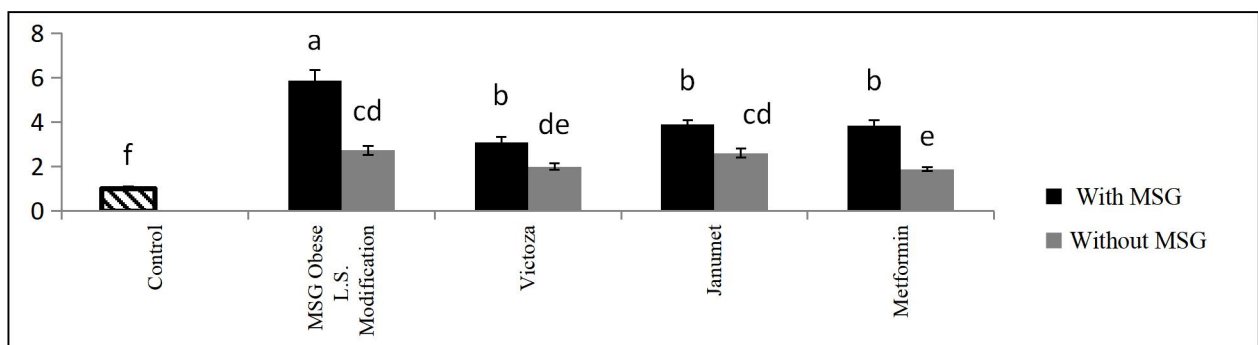
Analyzed data is mean of \pm SEM. Symbol (s) share the same superscript is not substantially different.

Figure 7 Glucagon serum levels (pg/mL) within different groups



Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

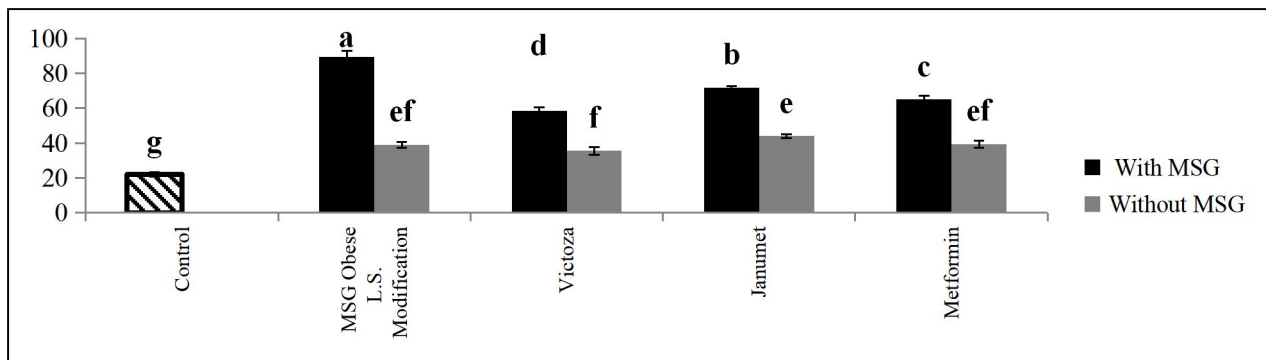
Figure 8 Calculation of HOMA-IR within different groups



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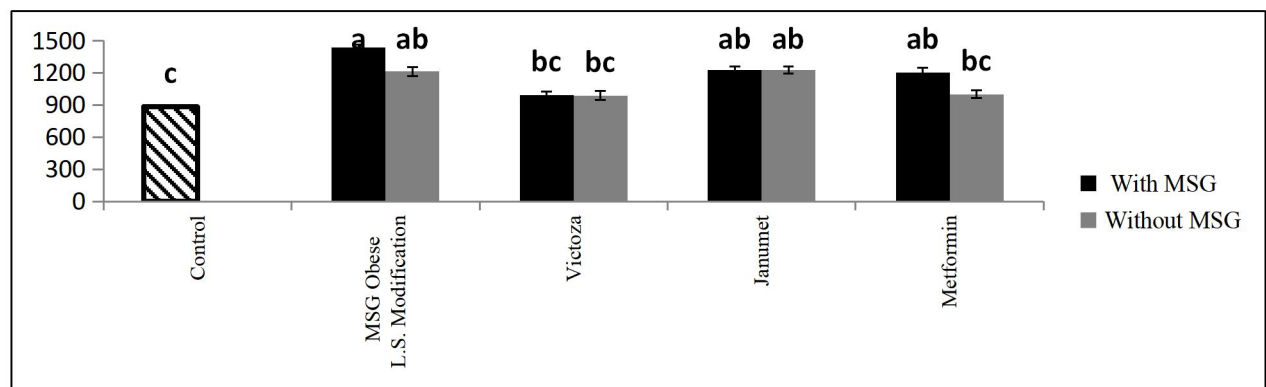
Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 9 Resistin serum levels (pg/mL) within different groups



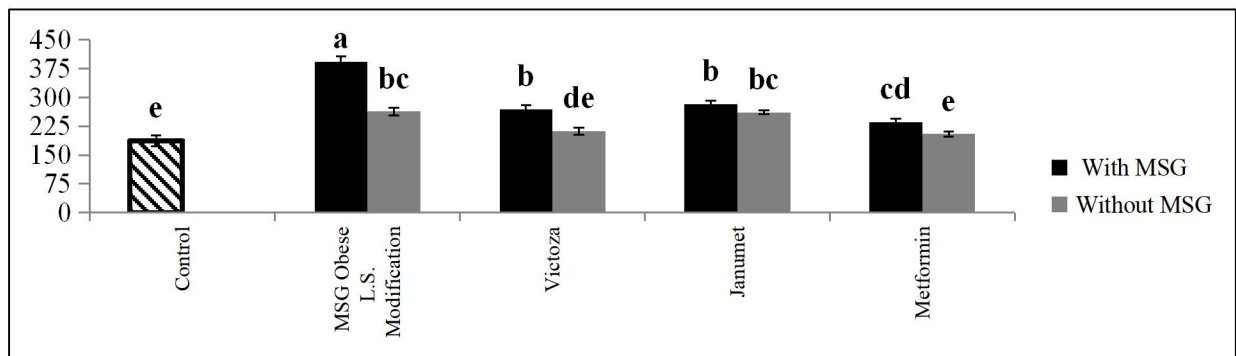
Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 10 Leptin serum levels (pg/mL) within different groups



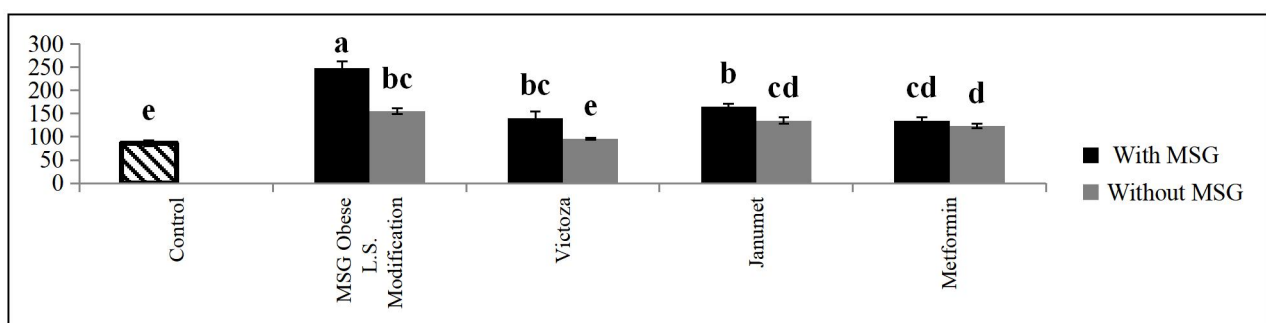
Analyzed data are expressed as mean \pm SE. Data with same superscript are not statistically different. SE, standard error.

Figure 11 Tumor necrosis factor-alpha serum levels (pg/mL) within different groups



Analyzed data are expressed as mean \pm SEM. Data with same superscript letters are not significantly different. SEM, standard error of mean.

Figure 12 Interleukin-6 serum levels (pg/mL) within different groups:



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Analyzed data are expressed as mean \pm SEM. Data with same superscript are not significantly different. SEM, standard error of mean.

DISCUSSION

The present study described the metabolic changes in male obese rats induced by MSG and their treatment using metformin, DPP4 inhibitor (Janumet), and liraglutide (Victoza). The effects of these drugs were observed on carbohydrate and lipid metabolism, and glucose hemostasis. The increase in the average bodyweight and levels of triacylglycerol, cholesterol was significant in MSG obese rats (subgroup B₁) that received MSG for 4 months as compared with the control group (subgroup A). These findings are consistent with those of Araujo et al. (47) who reported that MSG intake disrupted the energy equilibrium by disturbing the leptin-mediated hypothalamus signaling force, leading to obesity. In the MSG animal model, the affected regions included the hypothalamus responsible for adjusting body mass and energy metabolism (48). In addition, it leads to accumulation of lipids, resulting in lipotoxicity IR, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), and arterial hypertension (AHT) (49). The significant increase observed in subgroup B₁ in fasting blood sugar, HbA1c, and insulin levels is consistent with that of Bonnet et al. (50) who reported that MSG induced obesity, hyperlipidemia, hyperglycemia, IR and T2DM in rodents. Alterations in lipid and glucose homeostasis can adversely influence the sensitivity of insulin in obesity and other disorders, resulting in hyperglycemia and subsequent hyperinsulinemia due to increased levels of FFA along with elevated muscle plus lipid store and insulin resistance (51). The significant increase HOMA-IR and resistin levels in MSG obese rats (subgroup B₁) is consistent with that observed in the study by Lentferink et al. (52) who reported that obesity was detected using several IR predictors, which can be used to standardize IR screening. Elevated circulating levels of resistin have been observed in obese cases (53). Resistin release is often correlated with inflammatory reaction, IL-6 secretion, and hyperglycemia (54).

The marked increase observed in leptin levels in subgroup B₁ is in line with that reported by Sáinz et al. (55) who described that obese cases had high levels of circulating leptin. For individuals with insulin resistance and diabetes, increased rates of TNF- α , IL-1 β , IL-6, and C-reactive protein (CRP) were observed (56). Leptin, adiponectin, resistin, and retinol-protein binding 4 (RPB4) can further increase or decrease the insulin sensitivity (57).

Endogenous GLP-1 reduces the appetite, energy consumption, and gastric emptying; this explains the decrease in all treated groups and the reversal of bodyweight gain, reduced weight, and reduced food consumption observed with liraglutide (58).

In our study, Janumet treatment corrected hypertriglyceridemia, with a marked decrease in total cholesterol levels. GLP-1 inhibits the secretion of lipoprotein lipase and may decrease post-prandial hyperlipidemia (59). The reduced HbA1c levels in liraglutide against Janumet could be attributed to free pharmacological concentrations of liraglutide. It is reported that the increased expression of liraglutide GLP-1 receptor was several times higher than with DPP-4 inhibitors (60).

These results shown in Figs. 6 and 7 are in line with those of Gonzalo et al. (61) who reported that liraglutide

therapy resulted in reduced BMI and improved glucose and insulin. GLP-1RAs are similar to GLP-1 in activating the GLP-1 receptor, intensifying the secretion of insulin caused by nutrients (62).

Rats treated with Janumet and metformin in the second group without MSG had better results in fasting insulin levels than those in the first group with MSG. These results are in line with those of Moore et al. (63) who reported that lifestyle modification decreased bodyweight, FBS, and HbA1c, and improved insulin sensitivity. Results of Figs. 8 and 9 are in line with those of earlier GLP-1 clinical trials that reported that liraglutide markedly lowered the insulin resistance and increased the insulin secretion (64).

Liraglutide improves HOMA-IR of β -cell function, C-peptide and insulin levels (65). We noted that all subgroups without the MSG in the second group had significantly decreased levels of HOMA-IR and resistin ($p > 0.05$) when compared with same subgroups in the first groups (with MSG). These results show that lifestyle modification improve the resistance to insulin. Results of Fig. 10 are in line with those of Li et al, (66) who reported liraglutide decreased serum leptin levels in obese mice and patients with non-alcoholic liver steatosis by down-regulating SOCS3 and inhibiting the JAK/STAT pathway. Lifestyle modified rats (subgroup B₂) and all treated groups displayed reduced TNF- α levels ($p > 0.05$) when compared with subgroup B₁. However, subgroup C₂ and subgroup E₂ showed better results than other groups and attained normal levels with the control. The subgroup C₂ in the second group (without MSG) showed the best results among treated groups and attained normal rates with the control (Figs. 11 and 12). These results are in line with those of Lee et al, (67) who reported liraglutide reduced the levels of TNF- α , MCP-1, and IL-6, and generation and development of macrophages by suppressing the stimulation and phosphorylation of ERK1/2 and c-Jun N-terminal kinases. Janumet and metformin reduced the levels of TNF- α and IL-6 in the serum and IR-T2DM, results similar to those reported by Liliana et al. (68). The accumulation of pro-inflammatory cytokines and chemokines within the adipose tissue plays a major role in insulin resistance and T2D by obstructing inflammatory signaling and infiltration of immune cells in the adipose tissue (69).

CONCLUSION

Obesity is a result of an imbalance between biological, biochemical, medical, and metabolic factors that increases the risk of type 2 diabetes mellitus. Chemokines, such as IL-6 and TNF- α , play a crucial role in the development of obesity and insulin resistance. As MSG disrupts the metabolism with increased parameters, including leptin, HOMA-IR fatty acids, triglycerides, and pro-inflammatory adipokines, in addition to cytokine, lifestyle modification, is considered the first intervention choice for treating obesity, along with avoiding MSG-based diets. For those the change in lifestyle do not efficiently decrease the risk, pharmacological therapy should be considered. This study concludes that different anti-diabetic drugs improved the carbohydrate and lipid metabolism and levels of pro-inflammatory cytokines. Therefore, these drugs could be used to prevent the

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occurrence of T2DM in patients suffering from obesity and insulin resistance.

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