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Research Article

Orlistat and Metformin Effectively Reduce Pancreatic Dysfunction, Insulin Resistance and Blood Glucose Levels in Male Rats Experimentally Induced with Obesity

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Abstract

Background and Objective: Insulin resistance and glucose homeostasis are two conditions in which the pancreas is vital organ in their amelioration. Both insulin sensitivity and glucose metabolism can be strongly impacted by a high-fat diet (HFD). Insulin resistance and the pancreas are closely related when following a high-fat diet. Long-term HFD use may result in pancreatic dysfunction. **Materials and Methods:** Fifty adult male rats were randomly assigned into 5 groups: Control group: Rats were fed by standard diet with water *ad libitum*, OBS group: Rats were fed by high fat diet (26500.00" Kcal kg/calories/day) (for two times/day) successively for 30 days, OBS+ORS group: Rats induced OBS and then administered ORS at a dosage of (2 mg/kg) for 30 successive days, OBS+MEF group: Rats induced OBS and then concurrent treatment of MEF at a dosage of (70 mg/kg) for 30 successive days and OBS+ORS+MEF group: Rats induced OBS and then subsequent treatment of both ORS and MEF (1/2 hrs after the 1st dose) at a dosage for 30 successive days. **Results:** The HFD treated group showed significant elevation in body weight, serum glucose, insulin level, Hb1Ac and Homa-IR levels as compared to the control group. The pancreatic tissue of the same group showed a significant reduction in anti-oxidative markers as SOD and CAT and a significant reduction in MDA levels as compared to the control group. On the other hand, both groups treated with metformin and orlistat alone showed a significant attenuation of the aforementioned parameters. Remarkably, the combination of both metformin and orlistat showed superior beneficial protective properties over metformin or orlistat monotherapy. **Conclusion:** Both metformin and orlistat attenuated the glycemic disturbance and redox imbalance induced by HFD.

Key words: Pancreatic tissues, orlistat, metformin, oxidative stress, obesity, insulin resistance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pancreas, a critical organ in glucose homeostasis and insulin resistance play major roles in the complex link between dietary patterns and metabolic health. A high-fat diet (HFD) can have a considerable impact on markers of glucose metabolism and insulin sensitivity[1]. In the setting of a HFD, the pancreas and insulin resistance are intrinsically connected, playing critical roles in the development of metabolic disorders[2]. Prolonged exposure to a HFD can cause pancreatic dysfunction, which is defined by abnormalities in the pancreas structure and function. Inflammation, oxidative stress and changes in insulin-producing beta cells are all possible side effects of HFD[3]. These alterations affect the pancreas' capacity to produce insulin properly, which contributes to insulin resistance.

The HFD-induced insulin resistance develops when cells become less sensitive to insulin's activities. Insulin is essential for promoting glucose absorption by cells; when resistance develops, glucose levels stay elevated in the circulation. Insulin resistance is commonly caused by molecular changes such as inflammatory reactions, fat buildup and disrupted signaling pathways[4]. The interplay of the pancreas, insulin resistance and HFD severely affects glucose homeostasis[5]. Impaired insulin activity hinders cells from efficiently using glucose, resulting in high blood glucose levels. Persistent hyperglycemia is a sign of insulin resistance and can lead to metabolic diseases such as type 2 diabetes.

The HFD has been linked to an increased risk of oxidative stress, a condition in which the body's production of reactive oxygen species (ROS) outweighs its ability to neutralize them with antioxidants[6]. This imbalance can cause cellular damage and is linked to several chronic disorders. Several processes contribute to the relationship between a high-fat diet and oxidative stress. Superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) are important indicators in this context[6].

The SOD is an antioxidant enzyme that catalyzes the reduction of superoxide radicals. It plays a critical function in neutralizing ROS. The CAT is another antioxidant enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen, therefore reducing oxidative damage[7]. Changes in CAT and SOD activity is a cellular response to the HFD oxidative stress. The MDA is a marker for lipid peroxidation and oxidative stress. Elevated MDA levels indicate an imbalance between ROS formation and antioxidant defense, resulting in lipid peroxidation and cellular damage[8].

Metformin (MEF), commonly known as biguanide, is an oral medicine often used to treat type 2 diabetes mellitus. Its major action is to reduce hepatic glucose synthesis, improve peripheral insulin sensitivity and moderately reduce intestinal glucose absorption[9]. The MEF, as a first-line therapy for type 2 diabetes, does not increase insulin production but rather improves its efficacy. It has received attention for its possible antioxidant properties[10]. While the precise processes are not fully understood, numerous lines of evidence show that MEF may have antioxidant benefits via a variety of pathways including mitochondrial regulation, AMPK activation, Nrf2 induction, anti-inflammatory actions and improvements in glycemic control[11]. On the other hand, Orlistat (ORS) is a lipase inhibitor recommended to treat obesity. It works in the digestive tract by inhibiting the action of pancreatic and stomach lipases, reducing dietary fat absorption. The ORS is often used as part of a complete weight-loss program that includes dietary changes and exercise. While orlistat's primary function is not directly connected to antioxidant activity, its regulation of lipid metabolism, decrease in inflammation and changes in metabolic parameters may indirectly impact oxidative stress levels[12]. The aim of this study was to elucidate the potential synergistic protective effect of MEF and ORS against pancreatic dysfunction in HFD model.

MATERIALS AND METHODS

Study area: This study was carried out in Animal Physiology, Zagazig University on June, 2023.

Experimental animals: As 50 male albino rats "8 weeks age" were obtained from the Animal House of Faculty of Pharmacy, Zagazig University and weighing 150-180 g. The male rats were housed in hygienic cages with free access to regular standard feed and water for the normal control group. Meanwhile, the obese groups were given high fat diets and marked as (OBS) animals.

Ethical consideration: This experimental work was carried out under approval number (ZU-IACUC/2/F/62/2022). Based on the obtained ethical approval, anesthesia was obtained via administration of Ketamine-Xylazine at very low dosage to avoid any possible pain.

Experimental design: As 50 Male rats were divided randomly into 5 groups, 10 male rats/each group, experimental design as shown in Fig. 1. Treatment groups was divided into 5 group: Control group: Rats were fed by standard diet of normal equally balanced diet with water *ad libitum*, OBS group: Rats were fed by high fat diet (26500.00" Kcal/kg calories/day) (for two times/day) successively for 30 days, OBS+ORS group: Rats

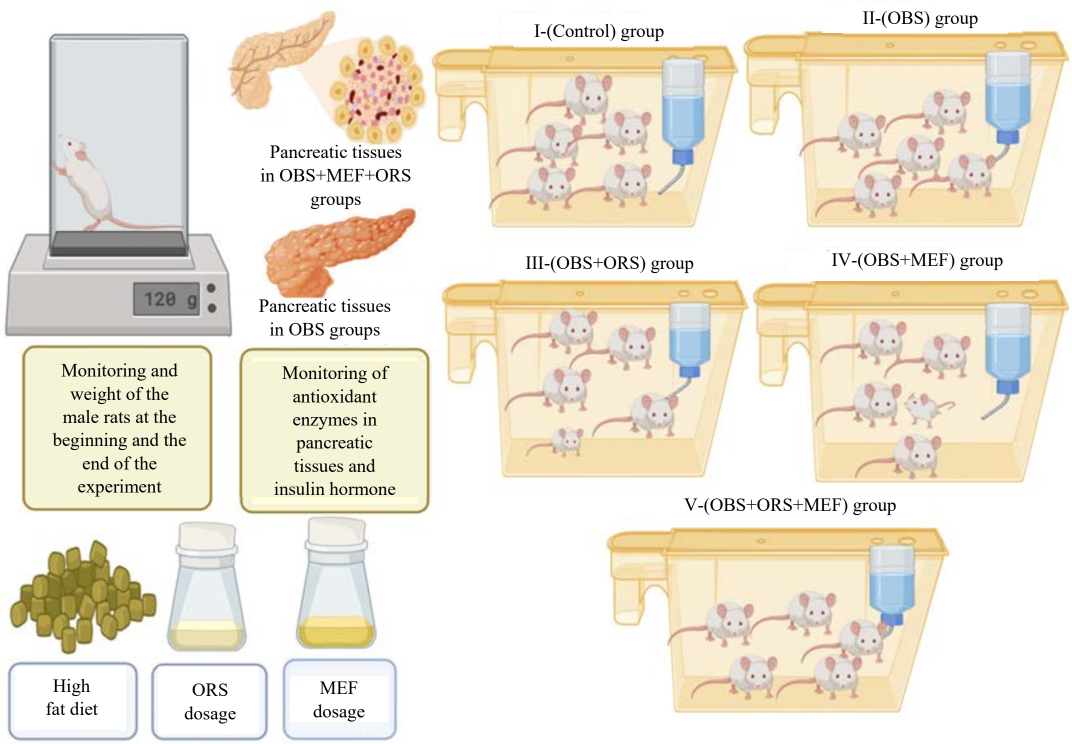

Fig. 1: Experimental design

were fed and induced OBS and then administration of ORS at a dosage of (2 mg/kg)[13] for successive 30 days, OBS+MEF group: Rats were fed and induced OBS and then concurrent treatment of MEF at a dosage of (70 mg/kg)[13] and OBS+ORS+MEF group: Rats were OBS and then subsequent treatment of both ORS and MEF (1/2 hrs after the 1st dose) at a dosage for successive 30 days.

Samples collection: Blood samples were withdrawn from the retro-orbital plexus and centrifuged at ~3000 rpm for about 5 min to get the serum samples. The serum samples were immediately persevered at -20°C for further biochemical assessment. The experimental animals were immediately decapitated after the anesthesia with xylene/ketamine (I.P) and the pancreatic tissues were harvested and were divided into three parts. One part was homogenized in phosphate buffer saline (PBS). Homogenate was subsequently used for biochemical analysis. The remaining pancreatic specimens were fixated in 10% buffered formalin for histopathological examination.

Biochemical investigation

Assessment of the oxidative stress markers: Small piece of the pancreatic tissues (~0.20 g) were homogenized with cold ice alkaline buffer and then get the supernatant for performing the antioxidant assays. Malondialdehyde (MDA) levels were measured according to Ohkawa *et al.*[14]. In accordance with Marklund and Marklund[15], superoxide dismutase enzyme activity (SOD) was measured. The breakdown rate of H_2O_2 was measured at 240 nm (Spectrophotometer SP-2200, Bioespectro)[16] and the CAT activity was calculated using Aebi's methodology. The results were expressed in units of (U/g). The activity of glutathione peroxidase (GPx) was assessed[17].

Determination the insulin hormone level: Insulin hormone levels were measured by automata (Elecsys, Roche Diagnostics) according to the manufacturers' protocols.

Histological assessment of pancreatic tissues: Fixed pancreatic tissues were processed by using Hematoxylin/Eosin staining. Photomicrographs of the pancreatic tissue samples were taken by light microscope digital camera (AmScope-MU1803-N105-USA) to view the stained slides according to Bancroft and Gamble[18].

Statistical analysis: The statistical data were expressed as (Mean±SE) by using One-way ANOVA. Significant value at $p \leq 0.05$. The CAT levels were assumed to be 2.17±0.56 in OBS induced group versus OBS+ORS+MEF with value 3.11±0.75.

At the power of 80% and the confidence level of 95%, sample size is 50 (10/each group). This statistical ratio was calculated by Snedecor and Cochran[19].

RESULTS

Changes in body weight: The body weight was changed greatly in different treated groups, the great elevation in body weight was recorded in OBS group. Meanwhile, the body weight reduction was recorded in both OBS groups treated with either ORS and/or MEF and the great amelioration was recorded in the group treated with both ORS and MEF treatment as shown in Table 1.

Effect on blood glucose level and insulin hormone: Blood glucose levels as recorded in Table 2 clarified the high elevation in blood glucose levels in OBS treated group. Meanwhile, the OBS group treated with either ORS and/or MEF induced decline in blood glucose levels and the more decline in blood glucose levels were recorded in group treated with both ORS and MEF treatments.

For insulin hormone levels as shown in Table 3, there was an increment in fasting Insulin hormone level in OBS treated group that indicates accumulation of insulin hormone levels without any real action and thus explains the increase in Insulin Resistance (HOMA-IR) levels , but all these parameters were ameliorated in other treated groups with both OBS and MEF treatments. Similarly HBA1C that was declined in treatment group, but it was highly increased in OBS treated group that indicates the recorded synergistic effects of both ORS and MEF treatment in amelioration of blood glucose levels, insulin hormone level and declining the insulin resistance in treated groups with both of treatments.

Oxidative damage marker: The pancreatic oxidative parameters of malondialdehyde (MDA) level, which is the final

Table 1: Body weight changes in control and different experimental groups

Groups	Change in body weight (gm)	
	1st week	4th week
Control	144.25±4.25^{b}	156.58±5.25^{b}
OBS	187.25±7.58^{a}	210.58±6.25^{a}
OBS+ORS	134.25±5.89^{c}	129.85±4.69^{c}
OBS+MEF	124.52±6.58^{d}	119.85±4.25^{d}
OBS+ORS+MEF	120.68±5.58^{e}	110.58±6.25^{e}

Data expressed as Mean±SE (n = 6) and values were significantly different at p<0.05

Table 2: Serum glucose level change (mg/dL) in control and different experimental groups

Groups	Changes in glucose level (mg/dL)	
	1st week	4th week
Control	75.25±5.68^{e}	74.28±4.69^{e}
OBS	250.36±4.25^{a}	298.69±5.69^{a}
OBS+ORS	149.25±6.25^{b}	135.69±5.25^{b}
OBS+MEF	136.28±4.58^{c}	128.85±3.58^{c}
OBS+ORS+MEF	100.58±2.69^{d}	90.25±3.69^{d}

Data expressed as Mean±SE (n = 6) and values were significantly different at p<0.05

Table 3: Serum insulin level, HOMA-IR and Glycated Hemoglobin (HbA1c) percentage in control and different experimental groups

Groups	Insulin (μIU/mL)	HOMA-IR	HbA1c (%)
Control	14.51±3.69^{e}	6.76±2.52^{e}	2.70±0.86^{e}
OBS	62.70±4.49^{a}	85.47±5.43^{a}	5.68±0.80^{ab}
OBS+ORS	52.96±4.91^{b}	36.74 ±4.73^{bc}	4.53±0.93^{b}
OBS+MEF	45.50±4.76^{c}	32.66±1.86^{c}	3.05±0.83^{cd}
OBS+ORS+MEF	33.73±4.44^{d}	14.85 ±1.69^{d}	2.56±0.92^{d}

Data expressed as Mean±SE (n = 6) and values were significantly different at p<0.05

Table 4: Superoxide dismutase (SOD), catalase (CAT) activities level and malondialdehyde (MDA) level in pancreatic tissues in both control and different experimental groups

Groups	SOD (U/mL)	CAT (U/mL)	MDA (ng/mL)
Control	32.73±1.94^{ab}	118.15±5.75^{ab}	50.93±4.47^{e}
OBS	17.53±2.23^{e}	40.73±4.13^{e}	105.78±4.80^{a}
OBS+ORS	20.13±2.84^{d}	92±3.55^{d}	80.87±4.56^{bc}
OBS+MEF	28.58±3.22^{c}	100.33±4.21^{c}	75.25±4.78^{c}
OBS+ORS+MEF	30.96±3.53^{b}	116.68±3.73^{b}	54.98±4.53^{de}

Data expressed as Mean±SE (n = 6) and values were significantly different at p<0.05

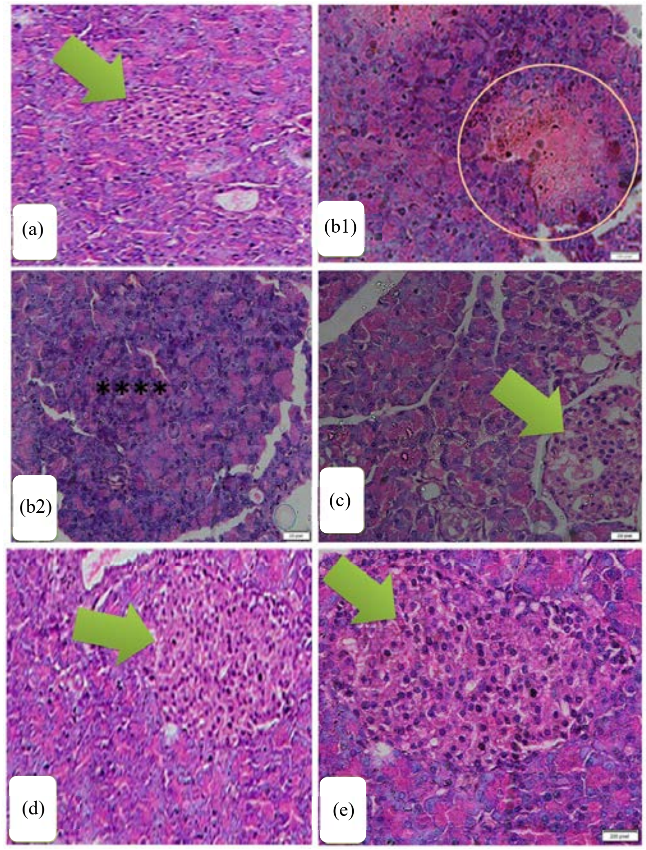

Fig. 2 (a-e): Histological sections of different treated groups, (a) Control group, (b1 and b2) OBS group, (c): OBS plus ORS treated group, (d) OBS plus MEF treated group and (e) OBS plus ORS and MEF group

(a) A cross-section of the pancreatic tissues showed normal appearance with normal appearance of mild sized pancreatic acini (Green arrow) (H&E ×400), (b1 and b2) High pancreatic toxicity and congestion with degeneration of the pancreatic tissues with clearing of their cytoplasm, vesicular nuclei and fibrosis around them (Orange circle) (H&E ×400), (c) Moderate sized pancreatic acini (Green arrow) with normal pancreatic architecture (H&E ×400), (d) High moderate sized pancreatic acini with clear pancreatic islets and granules (Green arrow) (H&E ×400) and (e) Normal pancreatic structure with high large sized pancreatic acini and clear islets and pancreatic granules (Green arrow)

marker of lipid peroxidation was significantly elevated in the experimentally induced obesity group after 30 days of treatment as recorded in Table 4. Both ORS and MEF declined this oxidative parameter marker as compared to the OBS and control groups, especially MDA level was significantly decreased in group treated with combination of both ORS and MEF.

Histological evaluation: The pancreatic tissues was normal in appearance in the normal control group (Fig. 2a) with normal appearance of mild sized pancreatic acini (H&E ×400). Figure 2b1,b2: OBS group showed high pancreatic toxicity and congestion with degeneration of the pancreatic tissues with clearing of their cytoplasm (H&E ×400). Figure 2c: OBS plus ORS treated group showing moderate sized pancreatic acini with normal pancreatic architecture (H&E ×400). Figure 2d: OBS plus MEF treated group showed high moderate sized pancreatic acini with clear pancreatic islets and granules (H&E ×400). Figure 2e: OBS plus ORS and MEF showing normal pancreatic structure with high large sized pancreatic acini and clear islets and pancreatic granules.

DISCUSSION

Obesity, a complicated health condition with several causes, requires evidence-based therapies. The ORS and MEF are two pharmaceutical medications that have been examined for their ability to treat obesity. The ORS, a lipase inhibitor, decreases the absorption of dietary fats, causing weight reduction. Its mode of action makes it an excellent supplement to lifestyle changes, particularly dietary adjustments and increased physical activity. The MEF, which is typically employed to treat type 2 diabetes, has received attention for its possible weight-loss benefits. While its specific processes in weight control are unknown, it is thought to affect appetite regulation and insulin sensitivity. The MEF has been shown in clinical research to provide moderate weight reduction, particularly in people with insulin resistance. This study aims to investigate the individual and combined effects of ORS and MEF on pancreatic function, insulin production and glucose homeostasis in experimentally induced obesity in rats. The findings of this study aim to provide valuable insights into the mechanisms underlying the therapeutic potential of the ORS and MEF combination, as well as a scientific foundation for potential interventions in the treatment of pancreatic dysfunction and associated metabolic abnormalities in obesity.

Obesity is evaluated in animal models using weight growth and/or an increase in body fat content. In contrast to the well-known BMI thresholds in humans, no standardized standards for obesity in animal models have been developed. Typically, in the experimental research, the degree of obesity is assessed by comparing the body weight of the experimental group, which is given a high fat, to control animals that develop normally when on chow or low-fat diet. In this study, a 4-week continuous HFD administration in the OBS group, resulted in a significant increase in body weight, which was consistent with the findings reported by Haleem and Mahmood[20].

In contrast, the administration of ORS resulted in a considerable drop in body weight when compared to the OBS group. This may be attributed to the fact that ORS inhibits fat absorption, resulting in a reduction in calorie intake from dietary fat. This adds to a negative energy balance, causing stored fat to be used for energy and, as a result, weight loss. Current results were consistent with existing literature or prior studies in the field[21]. Similarly, MEF showed a significant reduction in body weight. The MEF has also been shown to alter appetite management and energy expenditure, which adds to its potential impact on weight as it boosts insulin sensitivity, permitting better glucose utilization, which is frequently linked with insulin resistance. This, in turn, may contribute to a decrease in fat formation[22]. Interestingly, when ORS and MEF were combined, there was an additional substantial reduction in body weight relative to the groups treated with ORS or MEF alone. This shows a potential synergistic impact of combining ORS and MEF in moderating the rise in body weight caused by the HFD. These results highlighted the separate and collective benefits of ORS and MEF, supporting the hypothesis that their combination administration may provide greater efficacy in treating weight-related factors. Similarly, Hamza and Alsolami[13] confirmed the potent synergistic effects of ORS and MEF in reducing body weight.

The HFD has a significant impact on insulin dynamics, resulting in disturbances in glucose homeostasis. The complex link between a HFD and insulin dynamics includes a number of pathways that all lead to insulin resistance and metabolic dysfunction. In this study, rats fed on HFD showed significant changes in key metabolic markers such as serum insulin, HOMA-IR and HbA1c. Serum insulin levels were elevated, indicating a compensatory reaction to the increased metabolic demand caused by HFD[23]. The concomitant rise in HOMA-IR highlights the development of insulin resistance in response to long-term HFD intake. Furthermore, the measurement of HbA1c levels revealed an increase, indicating extended exposure to high blood glucose levels. This condition is compatible with the development of persistent hyperglycemia, which is a hallmark of poor glucose metabolism linked to high-fat diets. Current findings align with studies of Wang *et al.*[23] and Kurniawati *et al.*[24], this concurrence between our findings and previous research strengthens the credibility and validity of our model.

The ORS treatment to rats on HFD appears to have several impacts on key metabolic dynamics, altering measures such as blood glucose level, serum insulin, HbA1c and HOMA-IR. In this study, rats on HFD and treated with ORS alone showed a significant reduction in the blood glucose level, HB1Ac, serum insulin and HOMA-IR as compared to OBS group. The ORS may have an effect on blood glucose control via influencing fat absorption and insulin sensitivity[25]. Reduced absorption of dietary lipids may help with glucose regulation, resulting in lower blood glucose levels. The ORS decrease in fat absorption may have an impact on long-term glycemic management, as measured by Hb1Ac levels[26]. Moreover, by limiting the influx of dietary fats, it potentially mitigates factors contributing to insulin resistance, resulting in a more controlled serum insulin level. The improved insulin sensitivity contributes to a lower HOMA-IR, reflecting a potential amelioration of insulin resistance[27]. Our results align with that discussed in studies of Adeyemi *et al.*[28] and Khedr *et al.*[29]. The MEF, mostly recognized for its antihyperglycemic properties, is believed to help lower blood glucose levels. It works by increasing insulin sensitivity and lowering hepatic glucose synthesis, which improves overall glycemic management. In addition, MEF enhances insulin sensitivity and lowers hyperglycemia, helping to reduce HbA1c levels over time. The MEF does not specifically encourage insulin production, improved sensitivity may result in more efficient insulin use, potentially leading to changes in circulating insulin levels. In addition, MEF may help to reduce HOMA-IR by enhancing insulin sensitivity and lowering hyperinsulinemia, indicating a reduction in insulin resistance. Current results, came in accordance with previous literature[30,31].

The combination of ORS and MEF appears to have a synergistic effect in mitigating metabolic parameters, particularly those associated with glucose homeostasis. The observed significant reduction in blood glucose levels, HbA1c, insulin levels and HOMA-IR in the group treated with the combination of both drugs compared, suggest a comprehensive amelioration on metabolic dysfunction induced by HFD. Some studies warranted to elucidate the synergistic effect and optimize the therapeutic application of this combination in the context of metabolic disorders[32] which aligned with current findings.

The evaluation of antioxidant enzymes and oxidative stress indicators in HFD experimental model sheds light on how dietary composition affects redox balance state. The SOD, CAT and MDA are important indicators in this scenario. Rats subjected to HFD in OBS group should a significant decrease in the level of SOD and CAT and a significant increase in MDA levels. The significant reduction in SOD and CAT activity in OBS rats indicates an adaptive response to increased oxidative stress caused by the HFD. On the other hand, elevated MDA levels in HFD rats indicate an imbalance between ROS formation and antioxidant defense, resulting in lipid peroxidation and cellular damage. These findings matched with those reported in studies of Moraes-Souza *et al*.[33] and Zhang *et al*.[34]. The ORS unique methods for redox balance regulation may include a reduction in the formation of oxidative stress as a result of alterations in lipid metabolism. The ORS, by restricting dietary fat absorption, has the potential to lower substrate availability for ROS formation, hence relieving oxidative stress. Furthermore, ORS effect on the redox balance may involve the modification of antioxidant enzymes including SOD and CAT. The observed reduction in redox disturbance may indicate a restoration of antioxidant defense systems, which contributes to the overall preservation of cellular redox homeostasis. On the other hand, ORS significantly ameliorated the elevation in MDA level caused by HFD[35]. In our experiment, an observed reduction in redox imbalance in MEF treated rats, extending to the control of antioxidant enzymes, therefore strengthening the cellular defense systems against oxidative stress. Furthermore, MEF effect on cellular redox balance may include the activation of Amp-Activated Protein Kinase (AMPK), a cellular energy sensor[36]. The MEF-induced AMPK activation has been associated with better cellular stress responses and antioxidant defenses, which contribute to the relief of redox imbalances[36].

Interestingly, the combination of MEF and ORS may work synergistically, offering a dual strategy to combating oxidative stress[13]. This combination has the potential to target a variety of redox balance disruption mechanisms, including mitochondrial-derived ROS, lipid peroxidation and antioxidant enzyme activity regulation. The results in the combination group demonstrated an overall improvement in cellular redox equilibrium, restoring antioxidant defense systems and reducing oxidative damage caused by HFD-induced disruptions. Understanding the synergy between MEF and ORS in modulating redox equilibrium expands their potential therapeutic value beyond their basic activities.

Histologically, HFD treated rats showed congestion with degeneration of the pancreatic tissues with clearing of their cytoplasm, vesicular nuclei and fibrosis. Meanwhile, ORS treatment alone and MEF treatment alone showed a better architecture of the pancreatic tissue with moderate to high moderate sized pancreatic acini. The combination group showed a normal architecture of the pancreatic tissue with high sized pancreatic acini. Current finding came along with that proved in the literature. Understanding the synergies between MEF and ORS in the modulation of glycemic control and redox balance adds a valuable dimension to their potential therapeutic utility, extending beyond their primary functions.

CONCLUSION

The current study proved the synergistic amelioration effects of both ORS and MEF in reduction of body weight of OBS rats, with amelioration of blood glucose levels and insulin hormone levels with declining the insulin resistance and declining the oxidative injury in OBS male rats with elevation of antioxidant enzymes and improving the pancreatic structures than the pancreatic alterations that was resulted due to induction of experimental obesity, the obtained results greatly confirmed the current therapeutic approach with amelioration of antioxidant status of experimentally induced obesity.

SIGNIFICANCE STATEMENT

Current study proved the potent amelioration effects of both ORS and MEF in reduction of body weight, blood glucose levels, the insulin resistance and oxidative stress of OBS rats. Both treatments synergistically improved the pancreatic structures and achieved the potent therapeutic approach via amelioration of the antioxidant status of experimentally obesity.

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