ANIMAL RESEARCH

# Berberine attenuates olanzapine induced-metabolic syndrome

Marwa Salih Al-naimi, Huda Abdulbaki Rasheed, Hayder M. Al-kuraishy, Ali I. Al-Gareeb

## Abstract

**Objectives:** To elucidate the protective effect of berberine on olanzapine induced-metabolic syndrome.

**Methods:** This prospective experimental study involved thirty Sprague-Dawley male rats which were divided into three groups. Group A (n=10): Rats treated with distilled water, Group B (n=10): Rats treated with olanzapine, Group C (n=10): Rats treated with olanzapine plus berberine. The duration of the study was 8 weeks, baseline and follow up data were evaluated. Fasting blood glucose(FBG) total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL), Low density lipoprotein (LDL), atherogenic index of plasma (AI), fasting serum insulin level, insulin resistance, β- cell function and insulin sensitivity were evaluated.SPSS 20

**Results:** Olanzepine led to significant deterioration in gluco-metabolic profile compared with control P<0.01. Olanzapine plus berberine improved body weight, FBG, FSI, HOMA-IR and QUICKI compared with olanzapine P=0.0001.

**Conclusion:** Berberine attenuates olanzapine induced-metabolic via amelioration of gluco-lipid disturbances.

**Keywords:** Olanzapine, Metabolic syndrome, Berberine. (JPMA 69: S-88 (Suppl. 3); 2019)

## Introduction

Metabolic syndrome (MetS) is a group of disorders having at least three of the five medical situations including low high density lipoprotein (HDL), high triglyceride (TG), high blood glucose, high blood pressure (BP) and central obesity. MetS is associated with increased risk of cardiovascular complications and type 2 diabetes mellitus (T2DM).[1]

The precise mechanism of MetS is complex and not fully elucidated, but the initial predisposing factors include obesity, stress, sedentary lifestyle, diet high in fat, fructose or sucrose, high alcohol intake, sleep disorders and medications like psychotropic drugs.[2]

Olanzapine (OLZ) is a second-generation antipsychotic drug used in the treatment of schizophrenia and bipolar disorders. It acts through blocking serotonin receptors with little effects on dopamine receptors of the limbic system. OLZ therapy is associated with different side effects, including movement disorders, postural hypotension, insulin resistance (IR), seizure and MetS.[3]

Long-term therapy with OLZ is associated with accumulation of visceral fat which contributes into the development of IR, T2DM and cardiovascular complications.[4]

Department of Clinical Pharmacology, Medicine and Therapeutic, Al-Mustansiriya University, Baghdad, Iraq.

Correspondence: Hayder M. Al-kuraishy Email: hayderm36@yahoo.com

The mechanism of OLZ induced-MetS and weight gain is linked to central and peripheral disturbances. Blocking central serotonin and dopamine receptors lead to increase in the appetite that causes weight gain.[5] Preclinical and clinical studies illustrated that OLZ may cause MetS independent of weight gain but is linked to the increase in the visceral fat. Moreover, blocking effect of OLZ on the muscarinic receptor type 3(M3) may inhibit pancreatic insulin secretion leading to hyperglycaemia.[6]

Moreover, a study has showed that gut micobiota is involved in the development of visceral fat deposition and weight gain. Therefore, microbiota-free mice have 40% less visceral fat and are resistant to high-fat diet induced-obesity compared to the normal mice.[7]

More to the point, long-term OLZ therapy induces significant changes in gut microbiota owing to unknown mechanism, and elimination of this alteration by antibiotic and herbal agents may prevent or attenuate OLZ induced-MetS.[8]

Berberine (BRB) is an alkaloid herbal agent belonging to berberis used as a dietary supplement and for different therapeutic purposes. It has significant anti-obesity, anti-dyslipidaemic and anti-diabetic effects.[9] Hhypoglycaemic effect of BRB is insulin-independent and it is related to stimulation of glycolysis and inhibition of intestinal glucose absorption via inhibition of intestinal α-glucosidase enzyme. In addition, BRB improves lipid profile by increasing the expression of hepatic low density lipoprotein (LDL) receptors and modulation of proprotein convertase subtilisin/kexin type 9 receptors (PCSK9).[10]

Furthermore, BRB possesses significant anti-microbial activity against different microbes via inhibition of bacterial cell division and reduced gut microbiota induced-metabolic disturbances.[11]

The current study was planned to elucidate the protective effect of BRB on OLZ induced-MetS.

## Materials and Methods

This prospective experimental study was conducted in the Department of Pharmacology, College of Medicine, Mustansiriya University, Baghdad, Iraq, from March to June, 2018, and comprised thirty Sprague-Dawley male rats aged 2-3 months and weighing 100-250g that were brought from the International Centre of Medical Researches, College of Medicine, Mustansiriya University Baghdad-Iraq. The animals were isolated 3 rats in each cage that were placed at suitable room temperature and artificial 12/12hr light-dark cycle. They were given a week of acclimatisation without any intervention and with free access to normal chow pellets and water ad libitum. Permission was obtained from the institutional ethics committee in accordance with the Guide to the Care and Use of Laboratory Animal.[12]

After the acclimatization period, the rats were randomly divided into three equal groups. The study protocol for the induction of MetS was according to the method described by Weston-Green et al.[13]

Group A: rats were treated with distilled water 2.5 ml/kg/day orally for 8 weeks. Group B: rats treated with OLZ 3mg/kg/day (Film-coated tablets, 10mg, Accord Healthcare, UK) orally for 8 weeks. Group C: rats were treated with OLZ 3mg/kg/day plus BRB 300mg/kg/day (500mg tablets, USA) orally for 8 weeks.

Length was measured by graduated tape measure from nose to the anus (naso-anal length in cm). Rat bodyweight was measured by specific digital balance in gram. Body mass index (BMI) was measured as bodyweight (grams)/ length (cm)$^2$.

At the end of 8 weeks, the animals were kept on a fast for 12 hours and anaesthetised by using chloroform for rat decapitation. The blood samples were placed in labelled ethylenediaminetetraacetic acid (EDTA) containing tube centrifuged at 5000rpm for 5 minutes. The sera were stored at -20°C till analysis.

After 6 hours of fasting, fasting blood glucose (FBG) was measured by using glucometer. Serum total cholesterol (TC), TG and HDL were measured by enzyme-linked immunosorbent assay (ELISA) kit. LDL level was calculated by Friedewald equation.[14] Calculation of atherogenic index (AI) was calculated using the equation; AI = log (TG/HDL).[15] Fasting serum insulin level was measured by ELISA kit method (Rat insulin (INS) kit, Shanghai Yehua Biological Technology, China). Then IR, β-cell function and insulin sensitivity through Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as HOMA-IR = fasting insulin concentration (μIU/ml) x fasting glucose concentration (mg/dl)/405. β-cell function level was estimated by using the homeostasis model; HOMA-β= [fasting plasma insulin level (μIU/ml) x360]/ [fasting plasma glucose level (mg/dl)-63].[16] Insulin sensitivity was estimated using the quantitative insulin sensitivity check index (QUIICKI) equation: QUICKI=1/ [log (fasting plasma insulin (μIU/ml) +log (fasting plasma glucose (mg/dl))].[17]

Data was analysed using SPSS 20. Paired student t test was used to test the level of significance before and after intervention. Unpaired student t test was used to test the level of significance between two study groups. Analysis of variance (ANOVA) followed by Benferroni post-hoc Test was used to compare results of different groups. The level of significance was set at $p<0.05$.

## Results

Baseline data showed no significant difference in terms of bodyweight, lipid profile and glycaemic indices among the groups (Table-1).

After the 8-week intervention, bodyweight and BMI were significantly increased in Group B, and to a lesser extent in Group C compared to the control Group A ($p<0.01$).OLZ

Table-1: Baseline characteristics.

| Variables | Control (n=10) | OLZ (n=10) | OLZ+BRB | ANOVA |
|---|---|---|---|---|
| Length (cm) | 18.45±1.84 | 19.55±1.45 | 18.99±1.23 | 0.29 |
| Weight (g) | 190.78±10.76 | 188.90±11.84 | 191.87±11.81 | 0.84 |
| BMI(g/cm$^2$) | 0.55±0.01 | 0.54±0.04 | 0.56±0.03 | 0.33 |
| FBG (mg/dL) | 90.78±7.90 | 93.55±6.82 | 92.22±6.43 | 0.68 |
| FSI(?IU/ml) | 5.45±2.86 | 5.49±2.88 | 5.50±2.22 | 0.99 |
| HOMA-IR | 0.71±0.02 | 0.72±0.03 | 0.72±0.02 | 0.62 |
| QUICKI | 0.37±0.02 | 0.37±0.04 | 0.36±0.01 | 0.62 |
| TC(mg/dL) | 188.94±11.65 | 185.67±10.69 | 186.34±10.69 | 0.78 |
| TG(mg/dL) | 158.98±9.89 | 157.41±10.32 | 160.55±11.65 | 0.8 |
| HDL(mg/dL) | 51.66±6.98 | 49.86±6.74 | 53.22±5.11 | 0.5 |
| LDL(mg/dL) | 105.5±11.97 | 104.3±10.76 | 101.00±9.67 | 0.63 |
| VLDL(mg/dL) | 31.97±7.98 | 31.48±4.34 | 32.11±5.33 | 0.97 |
| AI | 0.12±0.015 | 0.13±0.033 | 0.12±0.029 | 0.63 |

Data isexpressed as mean±SD , one-way analysis of variance (ANOVA) test ,BMI: body mass index, FBG: fasting blood glucose, FSI: fasting serum insulin, HOMA-IR: homeostatic model assessment for insulin resistance, QUICKI: quantitative insulin check index equation, TC: total cholesterol, TG: total triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein, AI: atherogenic index, OLZ: olanzapine , OLZ+BRB: olanzapine-plus-berberine.

Table-2: Metabolic changes following eight weeks of treatment with olanzapine alone or in combination with berberine compared to the controls.

| Variables | Control (n=10) | OLZ(n=10) | OLZ+BRB | A | B | C | F Statistic | ANOVA |
|---|---|---|---|---|---|---|---|---|
| Length (cm) | 19.23±2.84 | 21.86±2.45 | 20.81±2.76 | NS | NS | NS | 2.42 | 0.1 |
| Weight (g) | 200.77±13.76 | 297.41±16.84 | 244.64±14.81 | 0.0001* | 0.0001* | 0.0001* | 101.46 | 0.0001 |
| BMI(g/cm$^2$) | 0.54±0.02 | 0.62±0.09 | 0.56±0.05 | 0.0007* | NS | 0.01¶ | 9.62 | 0.0007 |
| FBG (mg/dL) | 87.39±9.45 | 165.89±17.11 | 122.87±13.56 | 0.0001* | 0.0001* | 0.0001* | 81.91 | 0.0001 |
| FSI(µIU/ml) | 4.19±4.73 | 45.78±8.19 | 19.89±7.11 | 0.0001* | 0.0001* | 0.0001* | 94.51 | 0.0001 |
| HOMA-IR | 0.54±0.02 | 6.37±0.51 | 2.72±0.02 | 0.001* | 0.04¶ | 0.01¶ | 10496.73 | 0.0001 |
| QUICKI | 0.39±0.03 | 0.26±0.03 | 0.30±0.01 | 0.0001* | 0.0001* | 0.003* | 70 | 0.0001 |
| TC(mg/dL) | 189.94±10.66 | 285.58±19.47 | 201.58±12.64 | 0.0001* | NS | 0.0001* | 125.2 | 0.0001 |
| TG(mg/dL) | 168.98±8.42 | 357.41±28.09 | 210.49±14.78 | 0.001* | 0.001* | 0.001* | 218.34 | 0.0001 |
| HDL(mg/dL) | 51.87±6.55 | 39.11±3.74 | 40.66±7.89 | 0.0001* | 0.01* | NS | 12.2 | 0.0002 |
| LDL(mg/dL) | 105.5±11.97 | 104.3±10.76 | 101.00±9.67 | NS | NS | NS | 0.46 | NS |
| VLDL(mg/dL) | 33.79±8.32 | 71.48±8.34 | 42.09±8.96 | 0.0001* | NS | 0.0001* | 53.71 | 0.0001 |
| AI | 0.12±0.015 | 0.13±0.033 | 0.12±0.029 | NS | NS | NS | 0.46 | NS |

Data are expressed as mean±SD , one way analysis of variance (ANOVA) test and Post hoc-test ,NS: not significant , *p<0.01, p<0.05 ,BMI: body mass index, FBG: fasting blood glucose, FSI: fasting serum insulin, HOMA-IR: homeostatic model assessment for insulin resistance, QUICKI: quantitative insulin check index equation, TC: total cholesterol, TG: total triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein, AI: atherogenic index, OLZ: olanzapine , OLZ+BRB: olanzapine-plus-berberine.

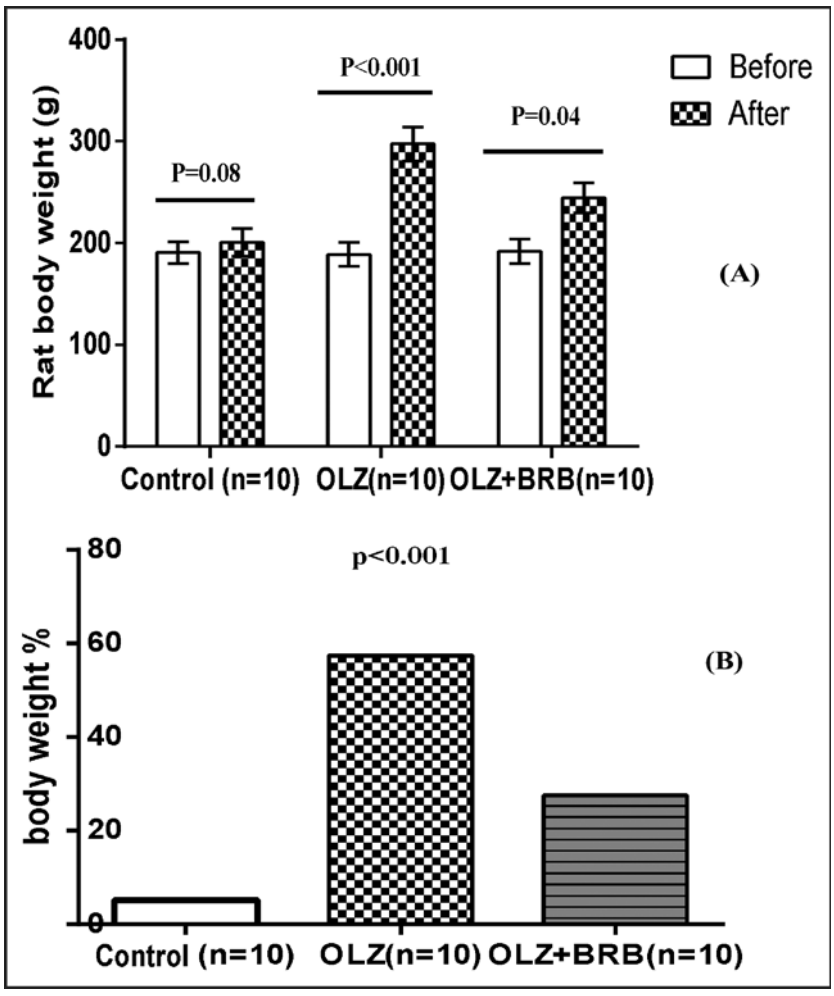


Figure: Bodyweight (A) and percent changes (B) at baseline and following 8 weeks of treatment in olanzapine group and olanzapine-plus-berberine combination compared to the controls.

led to significantly increased FBG (165.89±17.11mg/dL) compared with controls (87.39±9.45mg/dL) (p=0.0001). OLZ increased IR, reduced insulin sensitivity and raised insulin serum levels compared with controls (p<0.01). Also, OLZ induced significant deterioration of the lipid profile, increasing TC, TG, very low density lipoprotein (VLDL) and reducing HDL significantly compared with controls(p<0.01) without significant effects on LDL and AI (p>0.05).

OLZ+BRB showed significant increase in rats' bodyweight, increasing it to 244.64±14.81g compared with controls 200.77±13.76g (p=0.0001) without significant increase in BMI (p>0.05). FBG, fasting serum insulin (FSI), HOMA-IR and QUICKI also deteriorated in Group C compared to the controls (p<0.05). In lipid profile, only TG and HDL were affected compared to the controls (p<0.05). Group C illustrated significant differences in gluco-metabolic variables (p<0.05) except HDL, LDL and AI (p>0.05) compared to Group B (Table-2).

Bodyweight changes were non-significant before and after intervention in the control Group A (p=0.08). OLZ increased bodyweight significantly compared to the baseline value (p<0.001) while, OLZ+BRB

also increased bodyweight but to a slighter degree (p=0.04). Body weight percent changes were also noted (Figure-1).

## Discussion

The present study illustrated that OLZ led to significant induction of MetS in the experimental rats through increase in body weight, induction of dyslipidaemia, hyperinsulinaemia, reduction of insulin sensitivity and increment in peripheral IR. These findings correspond with earlier studies.[18]

Different studies discussed the potential mechanisms of OLZ-induced MetS. Peripheral IR and hepatic glucose overproduction lead to hyperglycaemia and glucose intolerance via induction of glucagon/insulin imbalance.[19] Additionally, chronic treatment with OLZ induces hepatic steatosis due to the inhibition of hormone sensitive lipase, reduction of fatty acid oxidation and induction of lipogenic and fatty acid synthase genes.[20] These findings might explain the gluco-lipid disturbances shown in the current study.

Furthermore, the present study disclosed significant effect of BRB in attenuation of OLZ-induced MetS through comparative reduction of bodyweight, IR, glycaemia indices and lipid profile. These results are in agreement with a study that showed significant effect of BRB in the prevention of OLZ-induced MetS[21] as BRB has a potential role in the prevention of fat accumulation, inhibition of high-fat-diet-induced weight gain and induction of weight loss.[22]

The mechanism of BRB against the development of MetS is related to the activation of adenosine monophosphate-activated protein kinase (AMPK), which activates glucose uptake and other catabolic processes with the potential to inhibit the anabolic process. Moreover, BRB activates brown adipose tissue activation and white-to-brown adipocyte conversion.[23]

A study confirmed the metabolic benefit of BRB via inhibition of lipid metabolism and glucogenesis, improvement of insulin sensitivity and prevention of IR.[24]

In spite of these effects, BRB in the present study illustrated non-significant amelioration on HDL, AI and LDL compared to the OLZ group which might be due to the short duration of the study and/or small doses of BRB to be able to demonstrate full effect on lipid profile.

As mentioned, OLZ leads to potential changes in gut microbiota which in part participate in the induction of MetS due to the involvement of gut-brain axis which is in the cornerstone of MetS.[25]

OLZ and diet-induced MetS lead to changes in gut microbiota with subsequent increase of intestinal permeability which increases the transmission of microbiota endotoxin. This endotoxin induces pro-inflammatory cytokines which are involved in IR and metabolic changes. BRB has anti-obesity effect through the modulation of gut microbiota and related endotoxaemia.[26] Therefore, BRB attenuates OLZ-induced MetS at intestinal and peripheral levels. But the anti-bacterial effect of BRB was not determined in the present study as it has been previously demonstrated.[27]

## Conclusion

BRB attenuated OLZ-induced MetS through the amelioration of insulin sensitivity and improvement of metabolic biomarkers.

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**Conflicts of Interest:** None.

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