ORIGINAL ARTICLE

EFFECTS OF METFORMIN ON THE WEIGHT OF HEALTHY AND STREPTOZOTOCIN-INDUCED DIABETIC ANIMAL MODEL

Akhtar Ali[1], Shehla Shaheen[1], Nisha Zahid[1], Urooj Zafar[3], Farah Ahmad[2], Lubna Farooq[3]

[1]Department of Pharmacology, [2]Department of Community Health Sciences, Ziauddin University Karachi-Pakistan

[3]Department of Pharmacology, Baqai Medical University Karachi-Pakistan

Background: Metformin was approved by FDA in 1994 for the treatment of type II diabetes mellitus. Due to its interaction with the AMP kinases enzyme system, it showed multiple effects on other organ systems of the body. Despite being the drug of the first choice for type II diabetes it became a popular drug for PCOS and currently, various trials are proving its anticancer effects. Though not approved by FDA it is also responsible for weight loss. The study aims to investigate the effect of metformin on the weight of healthy and streptozotocin-induced diabetic animal models. The study was performed at Animal House, Faculty of Pharmacy Ziauddin University Karachi. **Methods:** It was a pre-clinical experimental study conducted at Ziauddin University Karachi from August 2019 to December 2019. 36 male Albino rats were divided into 6 groups such with 6 rats in each group. Out of six three were healthy models (i.e., groups 1, 2, and 3) and the remaining three were diabetic models (i.e., Group 4, 5, and 6). Diabetes was induced in groups 4,5 and 6 by using the diabetogenic drug Streptozotocin. In Group 1 and 4, 0.5 ml normal saline was administered, 50 mg/kg (5 mg/100g) metformin diluted in 0.5 ml distilled water was administered in Group 2 and 5 followed by administration of 80 mg/kg (8 mg/100g) metformin diluted in 0.5ml distilled water in Group 3 and 6. the protocol was followed twice a day for 42 days. The weight of all the groups was calculated before and after drug intervention. **Results:** Metformin-induced weight loss was observed in healthy treated groups at both doses, however, there was no significant difference in weight loss in diabetic treated and untreated groups. **Conclusion:** Metformin showed weight lowering properties in healthy treated subjects**,** after induction of diabetes we found weight loss in diabetic untreated and treated groups which suggests that hyperglycaemia may cause weight loss but when metformin corrects the hyperglycaemic profile it does not lead to an increase in weight.

Keywords: Weight; Metformin; Streptozotocin; Animal model

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INTRODUCTION

It was 1920 when dimethyl biguanide a glucose lowering compound was extracted from *Galega officinalisa* plant which was then approved by FDA in 1994 for type II diabetes mellitus as "Metformin".[1] Later on it was highlighted that it does not possess only hypoglycaemic properties with lower toxicity profile, also it is helpful in weight loss. Due to once daily dosing, extended-release formulations and reduced risk of hypoglycaemia, metformin became the first choice of treatment for diabetes mellitus type II which is also denoted by a report, according to which there are 50 million prescriptions that include metformin not only for Diabetes mellitus but for several other uses in United states.[2,3] For diabetes mellitus proposed mode of action at tissue level is well explained in literature. However, at cellular level metformin interact with AMP Kinases enzyme system activation.[4] Interaction of metformin with AMP kinases enzyme system is responsible for its multiple therapeutic applications making it a useful drug to be used in PCOS, immune diseases, multiple cancers, as an antiaging agent and a weight lowering drug.[5–7]

Diabetes mellitus is characterized by chronic high blood glucose levels due to an imbalance in glucose uptake and insulin secretion. Due to decreased or absent insulin activity blood glucose level increases and leads the person to a metabolic disorder named Diabetes mellitus.[8] Diabetes mellitus has become an epidemic disease globally[9], and is nominated as one of the four diseases that are considered to be a priority among non-communicable diseases (NCD) as stated in the global report on diabetes by the world health organization[10]. As there is poor regulation of glucose levels in blood which leads to hyperglycaemia (high blood glucose level) showing various symptoms such as excessive urination, increased hunger, increased thirst, fatigue, and weight loss but as the condition aggravates with time, producing other symptoms like blurring in vision, infection recurrence and delayed wound healing.[11] Diabetes mellitus has been considered as a complication of obesity and it is always emphasized

that weight reduction in obese protects individuals from diabetes mellitus.[12] Balanced diet, regular exercise and weight control after diagnosis of diabetes mellitus have been associated with improved quality of life and better treatment response.[13] However, the patients with diabetes mellitus also show a tendency towards weight loss and muscle wasting.[14]

Metformin has shown promising weight lowering effects in obese individuals with and without insulin resistance and in women with PCOS. But in contrast when the patient is identified as diabetic (type II diabetes mellitus) and metformin is being prescribed as treatment of the first choice one must consider its all activities that can be beneficial or may be risky for patient. Specifically, if we consider weight loss as complication of diabetes mellitus than the question arises that either the metformin further potentiates weight loss in patients with type II diabetes mellitus or not. The current study is aimed to investigate the effect of metformin on weight of healthy and streptozotocin induced diabetic animal model.

MATERIAL AND METHODS

It was a pre-clinical experimental study conducted in Ziauddin University, Karachi from August to December 2019. We purchased 36 male Albino rats (from Agha Khan Animal Laboratory Karachi Pakistan), 9 weeks old, weighing about 300–400 g. The animals were divided into 6 groups (n=6 in each group). Out of six groups, three groups (i.e., group 1, 2 and 3) were healthy models and remaining three groups (i.e., Group 4, 5 and 6). were diabetic models Diabetes was induced in group 4,5 and 6 by using diabetogenic drug Streptozotocin.[15] We used Lidocaine topical gel to minimize needle pricking pain during the induction of diabetes and sample collection procedures. The ethical approval was taken from Animal Ethics Committee of Ziauddin University Karachi and protocol number 2018-003 was issued by the committee. During experiment animals were given free access to food and water, animals were kept at 20–22 ^{o}C temperature and 12-hour dark and light cycle was maintained furthermore, all animals were handled according to the 2010 guidelines of committee on animal research and ethics (CARE) for handling animals in experiments. Intervention was performed accordingly:

Healthy models:

Group 1: Control without intervention, only normal saline was administered

Group 2: Metformin(50mg/kg) dissolved in 5ml normal saline was administered

Group 3: Metformin (80 mg/kg) dissolved in 5ml normal saline was administered

Diabetic models:

Group 4: Without intervention, only normal saline was administered

Group 5: Metformin (50mg/kg) dissolved in 5ml normal saline was administered

Group 6: Metformin (80 mg/kg) dissolved in 5ml normal saline was administered

Dose Calculation:

50 mg / kg = 5 mg / 100g

80 mg / kg = 8 mg / 100g

Diabetes was induced in experimental animals by injecting 60 mg/kg (6mg / 100g) intraperitoneally.[16] On fifth day after administration of Streptozotocin fasting blood glucose levels were checked and compared with the controls to confirm the accuracy of procedure. After 15th day of streptozotocin administration animals were divided into the groups (i.e., Healthy Animals model 1, 2 and 3 and Diabetic Animal Model Group 4, 5 and 6).[17] In Group 1 and 4, 0.5 ml normal saline was administered, 50 mg/kg (5 mg/100g) metformin diluted in 0.5 ml distilled water was administered in Group 2 and 5 followed by administration of 80 mg/kg (8mg/100g) metformin diluted in 0.5 ml distilled water in Group 3 and 6.[18] Dose administration in all animals were performed through oral route by using 20g needle, the protocol was followed twice a day for 42 days. Weight of all the groups was calculated before and after drug intervention. Data was analyzed by using SPSS Version 20. Shapiro wilk test was applied to check the normality of data followed by Paired T test which was applied to identify the pre and post interventional changes in different groups. *p*-value of 0.05 was considered as significant at 95% confidence interval.

RESULTS

On the 2nd day of 60 mg/kg intraperitoneal streptozotocin administration in diabetic groups, FBS level of these groups (i.e., group 4, 5 and 6) were observed and was compared with negative controls. This comparison confirmed the induction of diabetes in group 4, 5, and 6 with highly significant (>0.001) *p*-value as shown in figure-1.

Pre and post interventional analysis of FBS is showed significant variation in the results. After the induction of diabetes mellitus in Group 4, 5 and 6, FBS levels were increased as shown in figure 1. While after administration of metformin to groups 5 (50 mg/kg) and 6 (80 mg/kg), FBS level was significantly (*p*-value 0.001) reduced in both groups when compared to positive controls (group 4). However, no change in FBS levels were observed in metformin treated healthy groups (Group 2 and 3) as represented in Table-1.

Significant decrease in weight has been seen in metformin treated healthy groups, i.e., Group 2 and 3 in comparison to the negative controls (Group 1) as shown in Figure-2. Weight reduction was also recorded in both treated and untreated diabetic groups (Group 4, 5 and 6) as depicted in figure 4. Moreover, when negative controls (Group-1) was compared with positive controls (Group 4), significant weight loss was observed in group 4, suggesting that induction of diabetes mellitus is significantly (*p*-value = 0.001) associated with weight loss in animals as shown in figure in 3. However, when positive control (group 4) was compared with group 5 and 6 no significant weight loss was seen in metformin treated diabetic groups (Group 5 and 6) (Figure 4).

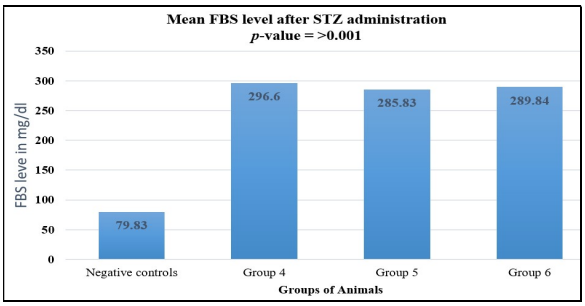

Figure-1: Comparison of glucose levels of Group 4, 5 and 6 with negative control group

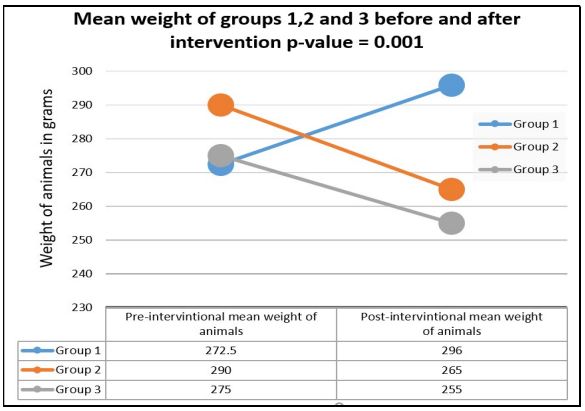

Figure-2: Mean weight of groups 1, 2 and 3 before and after intervention

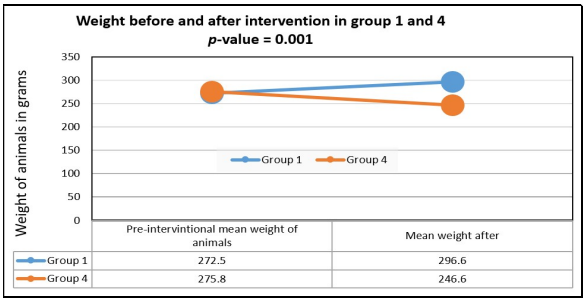

Figure-3: Weight before and after intervention in group 1 and 4

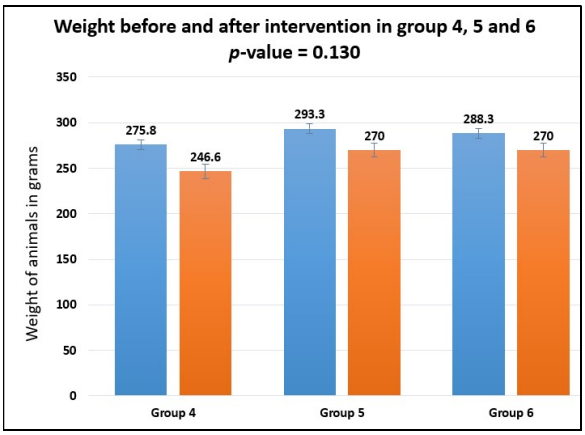

Figure-4: Weight before and after intervention in group 4, 5 and 6

Table-1: Pre and post interventional mean±SD FBS levels

Groups	Pre interventional Mean FBS level±SD	Post interventional Mean FBS level±SD	p-value
Group 1	79.83±8.7	81.6±10.3	0.729
Group 2	73.8±12.08	84.6±9.3	0.235
Group 3	79.84±12.9	81.5±7.9	0.764
Group 4	296.6±24.8	296.6±23.3	1.000
Group 5	285.83±8.9	109.6±7.9	0.001*
Group 6	289.83±3.71	115.5±7.9	0.001*

DISCUSSION

According to dose optimization, 60 mg/kg dose of streptozotocin induced the diabetes mellitus in group 4, 5 and 6 as shown in Figure 1. It is documented in literature that metformin in STZ induced diabetic models cannot decrease the glycaemic profile at lower doses.[19] However in another study at high doses metformin reduced the blood glucose level[20,21] which is in accordance to our results. The doses of metformin 50mg/kg and 80 mg/kg both significantly reduced the increased glucose level in diabetic rats (Group 5 and 6). The described mechanism of action of metformin for lowering the blood glucose level is decrease in hepatic glucose production and its intestinal absorption and increases insulin sensitivity[22] which shows that there is indirect activity of metformin in lowering the elevated blood glucose level. It is a well-established fact that metformin does not cause hypoglycaemia because it has no effects on insulin secretion, hence when the blood glucose is in normal range it does not cause any further change in glucose level.[23] The same findings were seen in metformin treated healthy groups that it neither caused hypoglycaemia, nor affected the blood glucose level at both doses, i.e., 50 mg/kg and 80 mg/kg.

Metformin is regarded as first line therapy in obese diabetic patients, it is not only responsible for controlling glycaemic profile in diabetics but also reduces the weight of patients.[24] According to literature metformin therapy has proved to reduce weight interfering with appetite regulatory pathways of brain metformin decreases the appetite that ultimately leads to weight loss.[25,26] Obese patients are suspected to develop T2DM but early treatment with metformin and life style modification may decrease the chances of development of disease.[27,28] There was significant association of weight loss with metformin therapy in our study. While evaluating animals in negative controls (Group 1) and in metformin treated healthy groups (Group 2 and 3) we found significant (p-value =0.001) weight loss as shown in Figure-2.

There was no association of dose of metformin in reduction of the weight in animals of group 2 and 3 (i.e., 50 mg/kg and 80 mg/kg) as illustrated in Figure-2. Weight lowering properties of metformin in various doses, i.e., 1500 mg and 2500 mg/day were also assessed by Harbone *et al*. in diagnosed cases of PCOS. Conversely to our study, metformin induced weight reduction was achieved at 2500 mg/day.[29] Obesity and T2DM are regarded as part of metabolic syndrome, which is associated with the decrease in the levels of adiponectins such as interleukin-6, tumour necrosis factor-alpha (TNF-alpha), resistin, leptin, angiotensinogen, and plasminogen activator inhibitor-1 (PAI-1).[30] Among them leptin is the only appetite suppressing hormone; its suppression in obese causes polyphagia, leads to the development of insulin resistance and ultimately manifested as T2DM.[31]

Furthermore leptin is documented to have inverse association with insulin resistance and it has been observed that after life style modification or treatment with pharmacological agents which tend to increase leptin , suppresses the appetite, participates in metabolism and causes weight loss.[32] On the contrary another study suggests that weight loss in T2DM is due to the decrease glucose transport inside the cells either as a consequence of deficient insulin levels or its actions on the body, Which drives the cells to utilize fats and proteins and hence leads to weight loss.[33,34] The results of our study are parallel with the findings of aforementioned observations, after induction of diabetes mellitus we found significant (p-value = 0.001) weight loss in positive controls (Group 4) when compared to the negative controls (Group 1) as illustrated in figure 3, however when metformin treated diabetic groups (group 5 and 6) were compared with positive controls (Group 4) we do not have any significant variation in weight of the animals as depicted in figure 4.

CONCLUSION

Metformin showed weight lowering properties in healthy treated subjects (Group 2 and 3) at both the doses, i.e., 50 mg/kg and 80 mg/kg, but not in a dose dependent manner. After induction of diabetes, we found weight loss in diabetic untreated (Group 4) and treated (Group 5 and 6) groups which suggests that hyperglycaemia may cause weight loss but when metformin corrects the hyperglycaemic profile it does not lead to increase in weight.

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Animal Handling: Committee of animal research and ethics 2010 guidelines were followed in all animal related procedures.

Ethical Approval: Study was approved by animal Ethics committee of Ziauddin University Karachi, Pakistan (Protocol No. 2018-003).

Conflict of Interest: There was no any conflict of interest in this study.

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AUTHORS' CONTRIBUTION

This work was carried out in collaboration among all authors. The concept of study, data analysis, drafting, and finalizing of the results were done by author AA. The article was critically reviewed by author SS. Finally reviewed and approved by author FA. Data collection and session organization was facilitated by NZ, and UZ, assisted by authors LF. All authors read and approved the final manuscript.

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Address for Correspondence:

Akhtar Ali, Department of Pharmacology, Ziauddin University Karachi, Pakistan
Email: akhtar.ali@zu.edu.pk/drakhtarali45@gmail.com