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Corresponding author:
Shehrina Nazmin, Department of Physiology, MH Samorita Hospital and Medical College, Dhaka.
E-mail: shehrinanazmin03@gmail.com

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Anti-diabetic effect of metformin combined with peanut (*Arachis hypogaea L.)* on streptozotocin induced diabetic rats

Shehrina Nazmin[1], Nayma Sultana[2]

1. Department of Physiology, MH Samorita hospital and Medical College, Dhaka.
2. Division of Basic Medical Sciences, School of Medicine and Health Sciences, University of Papua New Guinea.

Abstract

Background: Diabetes mellitus (DM) is a common metabolic disorder. Metformin is the initial drug of choice for treatment of type 2 DM. In many cases, metformin mono-therapy cannot effectively achieve the targeted glycemic control. However, metformin along with peanut (Arachis hypogaea L.) may reduce blood glucose level more effectively. **Objective:** To evaluate the anti-diabetic effect of metformin in combination with peanut on streptozotocin induced diabetic rats. **Methods:** This experimental study was conducted in the Department of Physiology, Sir Salimullah Medical College, in 2016. Forty (40) Wistar Albino male rats, 90-120 days old, weighing 225-240 g (initial body weight) were taken and divided into four groups containing 10 rats in each group, i.e. Non-diabetic group (ND), Streptozotocin induced diabetic group (STZ), Diabetic group treated with metformin (STZ-M) and Diabetic group treated with metformin and peanut (STZ-MP). Diabetic model was developed by giving single intraperitoneal injection of streptozotocin (50mg/kg body weight) to STZ, STZ-M and STZ-MP groups on day-1. In addition, STZ-M group received metformin (500mg/kg body weight) orally and STZ-MP group received both metformin and peanut extract orally (both 500mg/kg body weight) once daily in the morning for 21 days (day-4 to day-24). After measuring the final body weight, rats were sacrificed on day-25. To observe glycemic status, Fasting Blood Glucose (FBG), serum insulin levels were estimated and HOMA-IR was calculated. Statistical analyses were done by one way ANOVA and Bonferroni test as

applicable. **Results:** Mean FBG level and HOMA-IR were significantly ($p<0.001$) higher and serum insulin level and final body weight were significantly ($p<0.001$) lower in STZ group when compared to those of ND group. Whereas, FBG level and HOMA-IR were significantly ($p<0.001$) lower and insulin level and final body weight were significantly higher ($p<0.001$) in both STZ-M and STZ-MP groups in comparison to those of STZ group but more profound effects were found in STZ-MP group than those of STZ-M group. **Conclusion:** The present study revealed that, metformin combined with peanut was more effective to control glycemic status in diabetic rats than metformin alone.

Key words: Diabetes mellitus, Streptozotocin, Metformin, Peanut, Wister Albino Rats.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin actions or both[1]. It can be classified into type 1, type 2, gestational diabetes mellitus and specific types of diabetes due to other causes[2]. Throughout the world, the numbers of people with diabetes are increasing day by day due to population growth, aging, urbanization, stress and increasing prevalence of obesity and physical inactivity[3].

Streptozotocin is a nitrosourea glucosamine derivative and one of the most prominent diabetogenic chemical in diabetic research[4]. It is a broad spectrum antibiotic and alkylating genotoxic agent which possesses antibacterial, tumoricidal, carcinogenic and diabetogenic properties[5].

For the treatment of DM, different classes of anti-diabetic drugs are available. Among them, biguanide drug metformin is widely used and first line oral therapy for treatment of type 2 diabetes mellitus, particularly in overweight and obese people and those with normal kidney and liver functions[2,6]. It causes less weight gain and fewer hypoglycemic attacks than those of insulin and sulphonylureas[7]. The most frequent toxic effects of metformin are gastrointestinal disorders and occur in 20% of the patients. The most serious complication of biguanide use is lactic acidosis, which can be fatal[8].

DM is a progressive disorder which cannot be effectively managed with drug mono-therapy[9]. Mono-therapy with an oral hypoglycemic agent is often initially effective in controlling hyperglycemia but is associated with high secondary failure rates and required multiple therapies or exogenous insulin to achieve the target glycemic control[10].

Now a days, there has been increasing demand for the use of plant product with anti-diabetic activity due to low cost, easy availability and lesser side effects[11]. Among them, different nuts like walnut[12], peanut[13] etc. possesses anti-diabetic effect and used in the treatment of diabetes.

Arachis hypogaea L. known as peanut belongs to the family Fabaceae, have been valued for their high nutritional content throughout the world for many years. Peanut is a nutrient dense food, rich in plant protein that is high in arginine and a good source of mono and polyunsaturated fatty acids[14]. These unsaturated fatty acids improve insulin sensitivity and reduce risk of type 2 DM[15]. They also contain various vitamins like; á-

tocopherol, pyridoxine, niacin, folic acid, minerals like; copper, magnesium, potassium, calcium, zinc and several other bioactive phytochemicals like flavonoids, phenolic compounds like resveratrol and some phytosterol[14]. Flavonoid increases insulin secretion[16], decreases intestinal absorption of glucose[17] and has strong antioxidant property[18]. Moreover, resveratrol reduces hyperglycemia by increasing insulin sensitivity and â cell function[19]. Peanut consumption is relatively safe but causes food allergy in approximately 1% of the general population[20].

Recently, anti-diabetic effect of peanut was found in alloxan induced diabetic rats with significant improvement of FBG, HbA_{1c} levels and body weight[13]. Moreover, in another experimental study researchers found significantly lower levels of FBG, HOMA-IR and higher level of serum insulin in rats treated with conventional anti-diabetic drug metformin along with some natural products in comparison to that of metformin alone treated rats[21]. Therefore, the present study has been designed to observe the anti-diabetic effect of metformin in combination with peanut on streptozotocin induced diabetic rats. It is also expected that, the result of this study would make peanut acceptable among the people as a rich source of nutrients with high medicinal value for the treatment of diabetes mellitus.

Methods

This experimental study was conducted in the Department of Physiology, Sir Salimullah Medical College (SSMC), Mitford, Dhaka, from January to December 2016. The protocol of this study was approved by Institutional Ethics Committee (IEC) of SSMC.

Procurement and maintenance of animals[13,22]

Forty (40) healthy Wistar Albino male rats, 90 to 120 days old, weighing 225-240 g were used in this study. The animals were purchased from animal house of Department of Pharmacology, Jahangirnagar University, Savar Dhaka. All the animals were acclimatized for 14 days prior to intervention at 23±2Ë%C room temperature under 12 hours dark-light cycle. During this period, the animals had free access to food and water *ad libitum*. All the experiments and animal care were performed according to the international guidelines set in the 'Manual for Care and Use of Laboratory Animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). After 14 days of acclimatization, the total study period was 24 consecutive days.

Dose schedule

Fifty (50) mg/kg body weight powder of streptozotocin[23] (Sigma, USA) was dissolved in 0.1M freshly prepared citrate buffer (pH 4.5), five hundred (500) mg/kg body weight powder of metformin[23] (Square, Bangladesh) was dissolved in 5 ml/kg body weight of normal saline (NS) (Popular infusion limited, Bangladesh) and their solutions were prepared. In addition, five hundred (500) mg/kg body weight ethanolic extract of peanut[24] was prepared by modified Holden method[25].

Experimental design

At day-1, after measuring the initial body weight, all the rats were divided into four groups containing 10 rats in each group, i.e. Non-diabetic group (ND), Streptozotocin induced diabetic group (STZ), Diabetic group treated with metformin (STZ-M) and Diabetic group treated with metformin and peanut (STZ-MP). All the animals received basal diet for 24 consecutive days. In addition, STZ, STZ-M and STZ-MP groups received a single intraperitoneal injection of streptozotocin only on day-1 to produce diabetes mellitus. Again, STZ-M group received metformin orally and STZ-MP group received both metformin and peanut extract orally once daily in the morning for 21 days (day-4 to day-24). After 12 hours of overnight fasting, blood samples were collected from tail vein of all rats on day-1, day-4 and day-14 for estimation of FBG level. At the end of the study period i,e on day-25 morning, after measuring the final body weight and FBG level from the blood taken from the tail's

vein, all the animals were anaesthetized with the help of chloroform (30%). In deep anesthesia, 5ml of blood was drawn from left ventricle of heart. Then, the rats were sacrificed by decapitation. Serum insulin level was estimated in the laboratory of Department Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU). HOMA-IR was calculated according to the Matthews et al. (1985)[26] formula to estimate insulin resistance. The formula is-

HOMA-IR= fasting insulin (iU/ml) × fasting glucose (mmol/L) /22.5

Statistical analysis

All the data were expressed as mean ± SD. The statistical analyses were done by one way ANOVA and post hoc- Bonferroni test. In the interpretation of results, p value <0.05 was considered as significant.

Results

Initial body weight of ND, STZ, STZ-M and STZ-MP groups was almost similar and the differences were not statistically significant among all the groups. Whereas, final body weight was significantly lower in STZ group (p<0.001) in comparison to that of ND group. However, this level was significantly (p<0.001) higher in STZ-M and STZ-MP groups when compared to that of STZ group and also in STZ-MP group (p<0.01) than that of STZ-M group (Table I).

Figure 1 showed fasting blood glucose level in different groups of rats. Here, FBG level on day-4 was significantly (p<0.001) higher in STZ, STZ-M and STZ-MP groups and also on day-14 and day-25 only in STZ group in comparison to that of ND group. Whereas, on day-14 and day-25, this level was significantly (p<0.001) lower in STZ-M and STZ-MP groups when compared to that of STZ group and also in STZ-MP group (p<0.01)

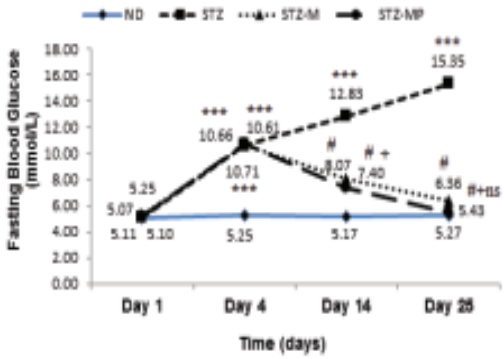

Figure 1: Fasting blood glucose level in different groups of rats. ND=Non-diabetic group, STZ=Streptozotocin induced diabetic group, STZ-M=Diabetic group treated with metformin (500mg/kg body weight), STZ-MP=Diabetic group treated with metformin and peanut (both 500mg/kg body weight). Each line symbolizes for mean±SD for 10 rats. ***=p<0.001, ^{ns}=p>0.05, compared to ND group, $^{\#}$=p<0.001, compared to STZ group and $^{+}$=p<0.01 compared to STZ-MP group.

Table I: Initial and final body weight of different groups of rats (n=40)

Parameters	ND (n=10)	STZ (n=10)	STZ-M (n=10)	STZ-MP (n=10)
Initial body weight (g) Day-1	233.76±3.68	232.51±3.72	234.44±3.88	234.91±4.28
Final body weight (g) Day-25	242.33±4.34	222.60±4.28***	229.80±4.34$^{\#}$	236.48±4.46$^{\#+}$

Results were expressed as mean ± SD. Statistical analysis was done by one way ANOVA and post hoc-Bonferroni test. ND=Non-diabetic group, STZ=Streptozotocin induced diabetic group, STZ-M=Diabetic group treated with metformin (500mg/kg body weight), STZ-MP=Diabetic group treated with metformin and peanut (both 500mg/kg body weight). ***=p<0.001, compared to ND group, $^{\#}$=p<0.001, compared to STZ group and $^{+}$=p<0.01, compared to STZ-MP group.

than that of STZ-M group, where it decreased towards the level of ND group and showed no statistically significant difference between STZ-MP and ND group.

Again, in this figure it has also been shown that, in STZ group, FBG level was significantly ($p<0.001$) increased from day-1 to day-4 and continued to rise on day-14 ($p<0.001$) and day-25 ($p<0.001$). Whereas, in SIZ-M and STZ-MP groups, this level was significantly ($p<0.001$) increased from day-1 to day-4 and then decreased gradually on day-14 and day-25 and tends to go towards the level of day-1, but only in STZ-MP group, this level reached the level of day-1 and showed no statistically significant difference between day-1 vs day-25.

The mean serum insulin level was significantly ($p<0.001$) lower in STZ group when compared to that of ND group. Whereas, this level was significantly ($p<0.001$) higher in STZ-M and STZ-MP groups in comparison to that of STZ group and also in STZ-MP group ($p<0.01$) than that of STZ-M group (Figure 2).

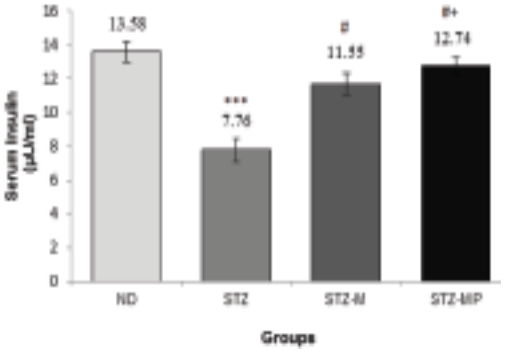

Figure 2: Serum insulin level in different groups of rats. ND=Non-diabetic group, STZ= Streptozotocin induced diabetic group, STZ-M=Diabetic group treated with metformin (500mg/kg body weight), STZ-MP=Diabetic group treated with metformin and peanut (both 500mg/kg body weight). Each bar symbolizes for mean±SD for 10 rats. ***=$p<0.001$, compared to ND group, $^{\#}$=$p<0.001$, compared to STZ group and $^{+}$=$p<0.01$, compared to STZ-MP group.

Again, mean HOMA-IR was significantly ($p<0.001$) higher in STZ group in comparison to that of ND group. Whereas, this value was significantly ($p<0.001$) lower in STZ-M and STZ-MP groups when compared to that of STZ group. Moreover, this value was almost similar and the differences were not statistically significant among STZ-M, STZ-MP and ND groups (Figure 3).

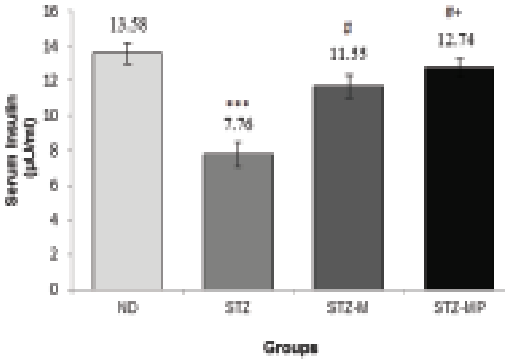

Figure 3: HOMA-IR in different groups of rats. ND=Non-diabetic group, STZ=Streptozotocin induced diabetic group, STZ-M=Diabetic group treated with metformin (500mg/kg body weight), STZ-MP=Diabetic group treated with metformin and peanut (both 500mg/kg body weight). Each bar symbolizes for mean±SD for 10 rats. ***=$p<0.001$, ^{ns}=$p>0.05$, compared to ND group, $^{\#}$=$p<0.001$, compared to STZ group and $^{\wedge}p>0.05$ compared to STZ-MP group.

Table II showed distribution of rats by different levels of fasting blood glucose at the end of the study period (day-25). In this study, 100% of rats in ND group had normal FBG level. Whereas, in STZ group 100% of rats were found diabetic. Again, in STZ-M group 30% rats showed normal FBG level, 60% of rats had IFG (impaired fasting glucose) and 10% rats were still diabetic. Moreover, in STZ-MP group, 90% of rats showed normal FBG level whereas, 10% rats showed IFG.

Table II: Distribution of rats by different levels of fasting blood glucose in all the groups of rats at the end of the study period on day-25 (n=40)

Groups	Fasting Blood Glucose Level					
	Normal	Total	IFG	Total	Diabetic	Total
	n (%)		n (%)		n (%)	
ND (n=10)	10 (100)		0 (0)		0 (0)	
STZ (n=10)	0 (0)	22	0 (0)	7	10 (100)	11
STZ-M (n=10)	3 (30)		6 (60)		1 (10)	
STZ-MP (n=10)	9 (90)		1 (10)		0 (0)	

Figures in parentheses indicate percentage. ND=Non-diabetic group, STZ=Streptozotocin induced diabetic group, STZ-M=Diabetic group treated with metformin (500mg/kg body weight), STZ-MP=Diabetic group treated with metformin and peanut (both 500mg/kg body weight).

Division of rats based on different levels of fasting blood glucose (according to ADA 2016)[1] -

- Normal: Rats having FBG level 3.6 to <6.1 mmol/L.
- IFG (impaired fasting glucose): Rats having FBG level 6.1 to <7.0 mmol/L.
- Diabetic: Rats having FBG level e”7.0 mmol/L

Discussion

In this study, the final body weight was significantly lower in STZ group in comparison to that of ND group. Whereas, this level was significantly higher in STZ-M and STZ-MP groups when compared to that of STZ group and also in STZ-MP group than that of STZ-M group. An almost similar finding was observed by other researchers by using honey along with metformin[27].

In the present study, all the STZ, STZ-M and STZ-MP groups of rats were found diabetic with higher fasting blood glucose levels on day-4 after administration of streptozotocin. And it was continued to increase in STZ group on day-14 and day-25. However, after treatment with metformin and peanut, FBG level decreased gradually from day-4 to day-14 and to day-25 in both STZ-M and STZ-MP groups, though the decrease was more in STZ-MP group, where it reached to the level of it’s pre-diabetic stage of day-1. Kumar et al. (2013)[24] made a similar observation. On the contrary, Sharma et al. (2010)[11] found no significant blood glucose lowering effect by using low dose (10mg/kg body weight) of some other traditional herbal medicine (ficus glomerata leaf extract) for a short duration of only 10 days in diabetic rats.

Again, hypoinsulinemia was found in STZ group, whereas, after treatment with metformin and peanut, insulin level was found to be higher in both STZ-M and STZ-MP groups of rats with more improvement was observed in STZ-MP group. Similar type of observation was also made by some other researchers[27]. On the other hand, Hadjikhani and Solati (2010)[28] did not find any significant change in serum insulin level in streptozotocin induced diabetic rats treated with different doses (0.1, 0.2, 0.5 and 1g/kg body weight) of alcoholic extract of walnut septum for 14 days. The researchers have suggested that, it might be due to the fact that, walnut septum has no effect on insulin secretion. Moreover, HOMA-IR was also found nearer to the value of ND group in both STZ-M and STZ-MP groups of rats after treatment with metformin and peanut. This finding was consistent with that of some other investigators[21].

At the end of this study period (day-25), all the rats (100%) in STZ group were found diabetic. Whereas, in STZ-M group, only 30% of rats had normal fasting blood glucose level, but a large number (60%) of rats couldn't achieve the normal FBG level and remain in IFG (impaired fasting glucose) state and 10% rats were still diabetic. But, in STZ-MP group, highest number (90%) of diabetic rats achieved the normal FBG level whereas, only 10% had IFG.

Diabetogenic dose of streptozotocin selectively damage insulin producing pancreatic â cells with subsequent inhibition of insulin synthesis and secretion from â cells. As a result, hypoinsulinemia and hyperglycemia developed[29].

Moreover, prolonged hyperglycemia alters insulin signaling pathway[30], reduces translocation of GLUT 4 to the plasma membrane[8] and decreases the sensitivity of target tissues to the metabolic effects of insulin and ultimately resulting in insulin resistance[30]. This is closely related to the toxic effects of lipid accumulation in tissues like skeletal muscle and liver[2]. Again, protein synthesis is reduced and proteolysis is increased in insulin deficiency, leading to increased muscle wasting and decreased body weight[8].

Anti-diabetic drug metformin reduces hepatic gluconeogenesis by activating AMP activated protein kinase (AMPK)[31], translocates GLUT 4 to plasma membrane[32], stimulates glucose uptake and fatty acid oxidation in muscle and liver, increases glucose utilization in peripheral tissues leading to increase in insulin sensitivity[33].

It has been suggested that, some active components of peanut like oleic acid[15], quercetin (a flavonoid present in peanut)[16] and resveratrol (a poly-phenol phytoalexin present in peanut)[19] stimulate insulin secretion from pancreatic â cells, inhibit the intestinal absorption of glucose[17], increases insulin sensitivity[19] and ultimately reduces the blood glucose level and lowers the risk of diabetic mellitus[15,34]. Treatment with peanut also improves body weight by increasing insulin secretion and sensitivity, which reduces proteolysis and prevents muscle wasting[13,24].

In the present study, streptozotocin induced diabetes mellitus was observed in Wistar Albino male rats as evidenced by their measured higher fasting blood glucose, lower serum insulin levels and decreased body weight. Again, higher level of HOMA-IR in the diabetic rats of the present study confirms presence of insulin resistance.

However, after treatment with metformin and peanut for 21 days, anti-diabetic effects were found in both groups of rats i,e in diabetic rats treated with only metformin and also in the diabetic rats treated with both metformin and peanut as evidenced by their measured lower FBG level, HOMA-IR value and higher serum insulin level and increased body weight. But more profound effects were found in rats who were taking combined treatment of both metformin and peanut, as proved by their measured FBG level. Higher number of rats with normal FBG level in this group further strengthens this statement. All these effects indicate that, metformin along with peanut act synergistically as an anti-diabetic agent. This synergistic effect of metformin and peanut may be due to, their combined action on increasing insulin sensitivity and also by increasing the secretion of insulin by peanut, as evidenced by their measured serum insulin level and HOMA-IR value. However, the exact mechanism involved cannot be elucidated from this study due to time and financial constraints.

Conclusion

From the result of this study, it can be concluded that, metformin alone has anti-diabetic effect, but the combined therapy of both metformin and peanut can synergistically reduce the blood glucose level towards the normal level and have more profound effects as an anti-diabetic agent. The active components of peanut may be responsible for these synergistic effects.

Therefore, the combination of peanut with metformin may be a valuable adjuvant therapy to achieve and maintain the target glycemic control.

Conflict of interest None

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