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RESEARCH ARTICLE

High Protein Diet Improves Biochemical and Metabolic Hormonal Profile in Alloxan-Induced Diabetic Rats

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A B S T R A C T

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level due to defective insulin secretion or action and is considered one of the major concerns to human health all over the world. Current study was planned to assess the effects of protein rich diet on body weight, glycemic control, insulin levels in hyperglycemic rats. For this purpose, high protein diet (45% or 55% fish protein rich in omega 3 fatty acids) was administered to alloxan-induced diabetic rats for a period of 28 days. Blood samples were collected for monitoring glucose level, serum insulin level and thyroid hormone levels. Alloxan administration resulted in reduced body weight, increased blood glucose, leptin while decreased insulin, amylin, glucokinase, thyroid stimulating hormone (TSH), triiodothyronin (T_3) and thyroxin (T_4) levels. High protein diet significantly restored body weight and showed significant anti-diabetic effect by reducing serum glucose and increasing serum insulin level in comparison to positive control (diabetic) group. Use of high protein diet also normalized serum amylin, leptin, GCK, T_3, T_4 and TSH. In sum, these results indicated the anti-hyperglycemic potential of high protein diet in alloxan-induced diabetic rat model.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level over prolonged period of time due to defective insulin secretion and action (Georgoulis *et al*., 2014, Rehman *et al*., 2018). It is considered to be one of the major concerns to human health all over the world (Ogurtsova *et al*., 2017). The global prevalence of diabetes has increased drastically since 1980. The total number of people with diabetes has increased from 106 million to about 422 million in the year 2014 rising from 4.7 to 8.5% of the adult population (Francis and Sudha, 2016).

Insulin and oral hypoglycaemic drugs such as, biguanides and sulfonylurea (Francis and Sudha, 2016) are being used to manage hyperglycemia and associated complications. However, satisfactory and effective therapy is yet to be available for treatment of diabetes mellitus. While complex interventions including dietary modifications can help in preventing the progression of impaired glucose tolerance in diabetes mellitus, limited evidence is available on the optimal dietary approach to control hyperglycemia (Schwingshackl *et al*., 2017). It is evident that reduced intake of total calories and weight loss are important in obtaining a good glycaemic control (Lih *et al*., 2015) however, the ideal proportion of the three main components of the food (protein, carbohydrate and fat) that should be recommended still remains unclear (Ajala *et al*., 2013).

Proper treatment, healthy eating habits and physical activity may help to ameliorate the diabetes symptoms (Evert *et al*., 2013). Stratagems of protein diet to maintain and achieve optimal body weight as well as normal blood glucose level have particular significance in managing the diabetes type 1 and type 2 (Campbell and Rains, 2015). Proteins present in diet not only reduce the postprandial concentrations of glucose, but also stimulate insulin secretion from β-cells of pancreas leading to a drop in elevated blood glucose concentrations (Campbell and Drucker, 2013). In case of diabetes, intake of proteins in diet up to 30% of total energy may help to maintain $HbA1_C$ (glycosylated hemoglobin) and glucose level in blood (Ajala *et al*., 2013). However, comparatively little evidence is present in literature regarding the impact of long-term

high protein diet intake on insulin sensitivity in diabetic subjects. The present study was designed to assess whether the high protein diet (fish origin) may ameliorate insulin sensitivity and thereby glycaemic control.

MATERIALS AND METHODS

Experimental design and induction of diabetes: Wister albino rats (180-250g body weight) were housed in animal house facility at Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad. The research trial was carried out by strictly following the guidelines on use and care of animals, approved by Institutional Bioethics Committee, University of Agriculture, Faisalabad, Pakistan. The rats were divided randomly into five groups (n=6), i.e., Group I: Control group receiving routine diet, Group II: Diabetic, alloxan monohydrate (140mg/kg body weight, intraperitoneally)-induced diabetic group receiving routine diet, Group III: Glib. (Glibenclamide, 10mg/kg b.wt., orally), alloxan induced-diabetic group treated with hypoglycemic drug glibenclamide, Group IV: High protein diet (HPD) 45%, alloxan-induced diabetic rats receiving 45% HP (fish) in diet, Group V: HPD 55%, alloxan-induced diabetic rats receiving 55% HP (fish) in diet.

Before induction of diabetes, the blood glucose levels of rats were determined. Diabetes was induced by administering single intraperitoneal injection of freshly prepared alloxan monohydrate, dissolved in normal saline @ 140mg/kg body weight (Federiuk *et al*., 2004). Fasting blood glucose level of each rat was measured from tail vein by using glucometer (OnCall ® *Ez II;* SN 303S0014E09) after 3, 7 and 10 days of alloxan injection to determine whether the animals had become diabetic or not. The rats exhibiting fasting blood glucose level ≥250 mg/dL were included. After ten days of alloxan administration, high protein (HP) diet i.e., 45 or 55% fish protein was provided to group IV and V respectively for a period of 28 days.

Physical parameter

Body weight and blood sampling: Body weight of each rat was recorded weekly throughout the experimental period. All the rats were anesthetized using chloroform and decapitated by cervical dislocation and blood samples were collected and centrifuged at 4000 rpm for 10-15 minutes to separate serum. The serum samples were stored at -20°C until analysis.

Determination of serum glucose, insulin, leptin, amylin and glucokinase (GCK) levels: Fasting blood glucose level was measured from tail vein by using commercially available glucometer (OnCall ® *Ez II;* SN 303S0014E09). Serum glucose, insulin, leptin, amylin and GCK levels were estimated by using commercially available diagnostic kits (Bioclin® Glucose Monoreagent diagnostic kit; Calbiotech Insulin ELISA® kit; rat-LEP ELISA kit: E-EL-R0582; rat-IAAP (Islet Amyloid Polypeptide) ELISA kit: E-EL-R2448; rat-GCK ELISA kit: E-EL-R0426).

Serum thyroid profile: Serum concentration of triidothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) was determined by using commercially available PISHTAZTEB diagnostic ELISA kits.

Statistical analysis: Results were expressed as mean ± SE. Data were subjected to ANOVA (analysis of variance) followed by DMR test to determine the statistical difference among the means of different treatment groups (Steel *et al*., 1997).

RESULTS

High protein diet restored the alloxan-induced decreased body weight: After experimental induction of diabetes by alloxan monohydrate, body weight reduced significantly ($P \leq 0.05$) in positive control group (diabetic) as compared to negative control and treatment groups. Treatment with high protein fish diet consisting of 45 and 55% restored body weight in treated groups (Fig. 1).

High protein diet showed significant glycemic control in alloxan-treated animals: Administration of alloxan monohydrate resulted in elevation of fasting blood glucose level that was maintained in positive control group throughout the experimental period. Daily treatment with glibenclamide and high protein diet over a period of 28 days significantly reduced ($P \leq 0.05$) fasting blood glucose level. There was no difference between 45 or 55% high protein diet groups (Fig. 2).

High protein diet restored serum glucose, insulin, leptin, amylin and GCK levels in alloxan-treated animals: A substantial decrease ($P \leq 0.05$) in serum glucose level (Fig. 3) and noticeable increase ($P \leq 0.05$) in insulin level (Fig. 4) were observed in treated groups as compared to positive control. Alloxan administration significantly raised ($P \leq 0.05$) the serum leptin level which were restored to normal in rats receiving HP diet (Fig. 5). A substantial decrease ($P \leq 0.05$) in amylin and GCK levels was also observed in alloxan-induced hyperglycemic rats in comparison to negative control group whereas, glibenclamide and HP diet treatment restored the normal serum amylin and GCK levels (Fig. 6 & 7).

Effect of high protein diet on thyroid hormone profile: A noteworthy reduction ($P \leq 0.05$) in serum T_3, T_4 and TSH levels was observed in diabetic group as compared to negative control group while HP diet treated rats showed a significant improvement in thyroid hormone profile (Fig. 8).

DISCUSSION

The three main components for managing diabetes include healthy eating habits, regular exercise and pharmacotherapy. The most challenging component of this strategy is to recommend what to eat. Therefore, nutritional therapy plays a vital role in diabetes management. American Diabetes Association recommends that every person suffering from diabetes mellitus must be actively engaged in diet planning and self-management with the guidelines of a nutritionist (Inzucchi *et al*., 2012). The present study explored the effect of high protein (HP) diet on body weight, glycaemic control and different metabolic hormones in diabetic rat model.

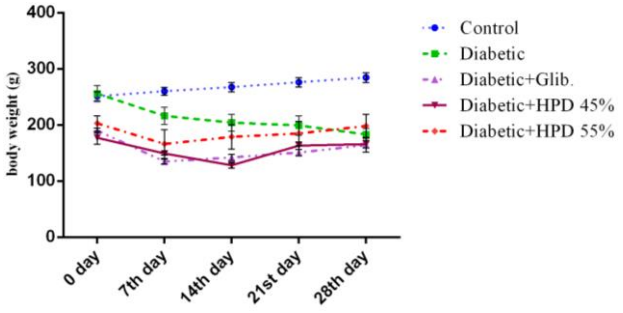


Fig. 1: Trend line for body weight (g ± SE) of negative control, positive control, glibenclamide and high protein diet treated groups at different days of treatment.

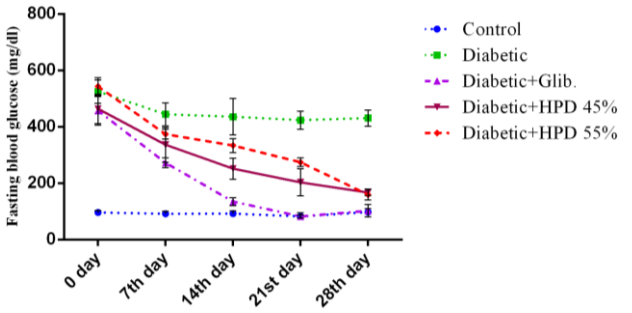


Fig. 2: Trend line for fasting blood glucose level (mg/dl ± SE) of negative control, positive control, glibenclamide and high protein diet treated groups at different days of treatment.

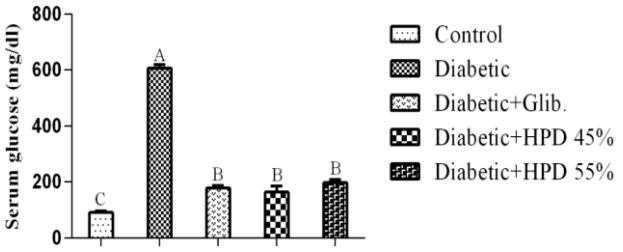


Fig. 3: Effect of HP diet on serum glucose concentration (mg/dl) in alloxan-induced diabetic rats.

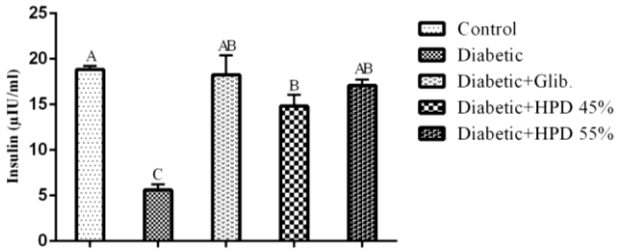


Fig. 4: Effect of HP diet on serum insulin (µU/ml) levels in alloxan-induced diabetic rats.

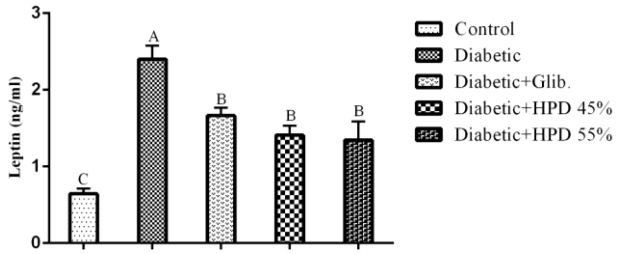


Fig. 5: Effect of HP diet on serum leptin (ng/ml) levels in alloxan-induced diabetic rats.

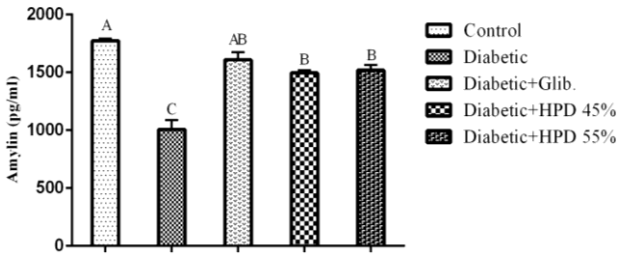


Fig. 6: Effect of HP diet on mean serum amylin (pg/ml) in alloxan-induced diabetic rats.

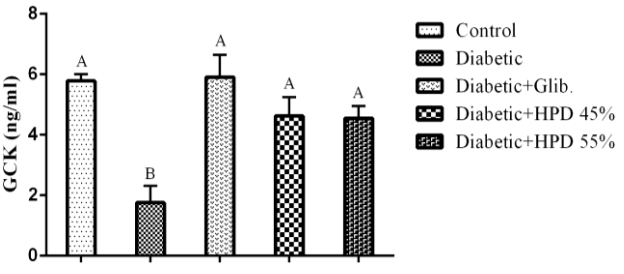
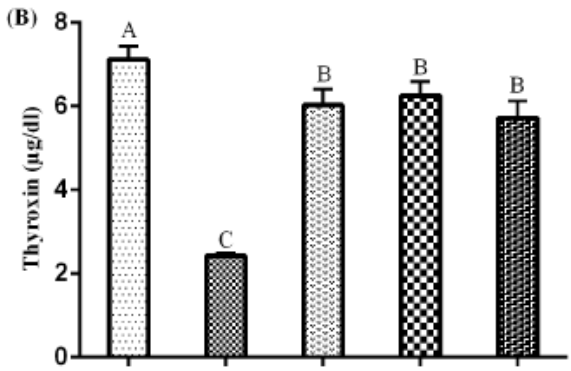


Fig. 7: Effect of HP diet on serum GCK (ng/ml) levels in alloxan-induced diabetic rats.



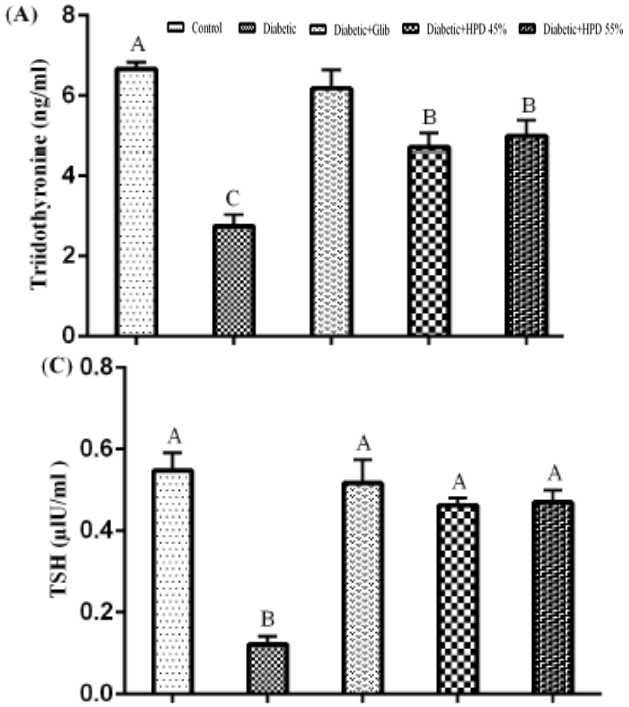


Fig. 8: Effect of HP diet on metabolic hormonal profile (A) Serum T_3 (ng/ml) levels (B) Serum T_4 (µg/ml) levels (C) Serum TSH (µIU/ml) in alloxan-induced diabetic rats.

Alloxan-induced reduction in body weight could be attributed to the structural protein degradation as diabetes is associated with different biochemical derangements including increased glycogenolysis and lipolysis leading to tissue protein loss and muscle wasting (Nagy *et al*., 2015). Treatment with high protein diet consisting of 45 and 55% fish might have prevented protein and fat catabolism thereby restoring body weight of both treated groups. This finding is consistent with a previous study conducted by Mustafa *et al*. (1993) in which carbohydrate free diet was used to feed the hyperglycemic rats and a strong relationship between carbohydrate free diet and weight gain was observed as treatment group showed a steady increase in body weight in comparison to positive control group.

Continued elevation in fasting blood glucose level in alloxan-treated animals suggested successful induction of hyperglycemia. Daily treatment with glibenclamide and high protein diet over a period of 28 days significantly decreased ($P \leq 0.05$) fasting blood glucose level. This finding indicated congruence with previous study in which a decline in fasting blood glucose level was observed after the consumption of cod protein in normoglycemic rats (Lavigne *et al.,* 2000). A significant decrease ($P \leq 0.05$) in mean serum glucose concentration and significant increase ($P \leq 0.05$) in serum insulin level were also observed in all treatment groups in comparison to diabetic group. Previous researchers (Elberry *et al*., 2015; Jayachandran *et al*., 2018) have also described decline in mean insulin level in diabetic rats as observed in the current experiment. In another study, it was observed that serum insulin level was higher in diabetic rats fed on high protein diet compared to those receiving normal diet (Eizirik *et al.,* 1986). It is hypothesized that high protein diet might exert anti-hyperglycemic effect by reduction of oxidative stress in pancreatic β-cell or recovery/regeneration of damaged β-cells through availability of essential amino acid.

Leptin, a vital hormone derived from adipose tissue, plays a significant role in various pathways prompting the risk of diabetes and cardiovascular diseases (Patel *et al*., 2016; Katsiki *et al*., 2018). In current study, diabetes induction by alloxan significantly increased ($P \leq 0.05$) leptin concentration in serum, while a significant decrease in serum leptin level ($P \leq 0.05$) was observed in high protein diet group. This reduction in leptin level in treated groups could be correlated with increased insulin secretions in high protein diet treated groups consistent with a previous study (Kulkarni *et al*., 1997) suggesting an inverse relationship between insulin secretions and leptin levels. Some studies concluded no association between diabetes and serum leptin level (Maahs *et al*., 2009) while other studies have reported a significant positive relationship between diabetes and serum leptin level (Soderberg *et al*., 2007; Welsh *et al*., 2009). However, an inverse relation between serum leptin level and diabetes has also been reported (Sun *et al*., 2010).

Amylin, also recognized as amyloid polypeptide of islet, is a neuroendocrine hormone that is co-localized and co-secreted with insulin from β cells of pancreas (Zhang *et al*., 2016). Amylin contributes to blood glucose homeostasis along with insulin and glucagon secreted from pancreatic islet (Zhang *et al*., 2016). The insulin and amylin function as a pair of synergistic partner genes that are co-expressed by a common promoter and regulate blood glucose level by complex neuronal as well as endocrine pathways. Any abnormality in secretion or action of amylin negatively affects functioning of pancreatic islet and regulation of glucose by β cell dysfunction and amyloidosis leading to diabetes (Kahn *et al*., 1999). In present, study significant decrease ($P \leq 0.05$) in amylin level was observed in diabetic group in comparison to negative control group whereas; all other treatment groups exhibited a significant rise ($P \leq 0.05$) in amylin level. These results are supported by a previous research in which it was observed that induction of diabetes by streptozotocin in rats resulted in loss of ability to secrete amylin while arginine and glucose treatment stimulated the release of both amylin and insulin in a parallel pattern (Ogawa *et al*., 1990).

In mammals, glucokinase (GCK) is mostly available in liver and pancreas and it represents 95% activity of hexokinase, an enzyme that plays an important role in insulin independent uptake and utilization of glucose (Adewole and Ojewole, 2009). In β-cells of pancreas, GCK acts as glucose sensor to modify the secretion of insulin (Baldini *et al*., 2016). Hyperglycemia causes a reduction in efficiency of GCK function leading to decreased sensitivity of glucose mediated insulin secretion response of β-cells (Zelent *et al*., 2005). The results of present study revealed similar pattern as level of serum GCK was reduced significantly ($P \leq 0.05$) in alloxan-induced hyperglycemic rats as compared to negative control group whereas glibenclamide and high protein diet treatment restored the normal serum GCK level. The decrease in serum GCK levels in hyperglycemic rats might be due to hypo-insulinaemia, increased degradation or reduced synthesis of GCK resulting from oxidative stress in diabetes mellitus (Matschinsky and Magnuson, 2004). However, the ability of high protein diet to significantly restore ($P \leq 0.05$) the serum GCK to optimal level would seem to suggest insulin-releasing potential of high protein diet in rats of treated groups.

Thyroid hormones play a vital role in development and normal growth of a maturing individual. It has been suggested that serum thyroid hormones are negatively affected in diabetes (Joffe and Distiller, 2014). In present study a significant reduction ($P \leq 0.05$) in serum T_3, T_4 and TSH levels in positive control group as compared to negative control groups while HP diet treated diabetic rats presented a significant improvement in thyroid hormone profile. These findings are supported by the previous studies (da Silva Teixeira *et al*., 2016) where a significant decrease in level of serum thyroid hormone was observed in experimentally induced hyperglycemic rats. This decline in serum thyroid hormone concentration might be due to inhibition of de-iodination of T_4 and other partially iodinated threonine into T_3 in diabetic rats.

Conclusions: Diet plays key role in promoting health through metabolic homeostasis. It is also directly or indirectly related to the prevalence many chronic diseases including diabetes. High protein diet is particularly important to maintain good glycemic control and an ideal body weight. Results revealed that high protein diet lower the blood glycemic condition by improving the level of

serum insulin level in comparison to diabetic group and exhibited a significant rise ($P \leq 0.05$) in amylin level by significantly restoring ($P \leq 0.05$) the serum GCK to optimal level that would seem to suggest insulin-releasing potential. Results also revealed that there is significant reduction ($P \leq 0.05$) in serum T_3, T_4 and TSH levels in positive control as compared to all other groups and HPD treated diabetic rats showed a significant improvement in thyroid profile. Over all our results revealed that high protein diet improves insulin, amylin, GCK, T_3, T_4, TSH hormones and lowers the blood glucose level.

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Authors contribution: The work is a product of the intellectual environment of the whole team; and all the members have contributed in various degrees in designing the study, developing the methodology, performing the analysis and writing the manuscript.

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