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'Betazen' Herbal Formulation, Ameliorates Experimentally Induced Metabolic Disturbances in Insulin Resistant Rats

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Abstract

The present study was designed to evaluate anti-diabetic activity of ayurvedic formulation 'Betazen' in experimentally induced insulin resistant rats. Wistar rats were being rendered insulin resistant by administration of dexamethasone (2 µg/day s.c), along with high fat-high sugar (HFHS) diet (i.e. vanaspati ghee and fructose enriched) for 42 days. Animals with fasting blood glucose levels (BGL) of more than 150 mg/dl were selected, divided into different groups and administered 2.7 mg/kg pioglitazone, 45 mg/kg, and 90 mg/kg betazen herbal formulation (BHF) respectively, suspended in 1% CMC for 30 days p.o. At the end of the study, animals were sacrificed; blood was collected and used for estimation of various biochemical parameters and HbA_{1c}. Whereas, glycogen content in the liver and gastrocnemius muscle, glucose uptake in hemi-diaphragm and antioxidant biomarkers in liver were also estimated. Pancreas were subjected for histopathological studies. The results obtained from the present study exhibited a significant dose dependent decrease in elevated fasting BGL, lipids (TC, TG, HDL, LDL and VLDL), HbA_{1c} and MDA levels. Further, there was a significant ($p<0.001$) increase in glycogen content, glucose uptake, HDL, and antioxidant biomarkers viz.; GSH, SOD and CAT. These experimental results highlighted the antioxidant, anti-glycation and anti-hyperglycemic potential of BHF on insulin resistance. It further exhibited an improvement in dyslipidemia, insulin sensitivity, and glycogen content. Hence, BHF could be a promising future herbal treatment for insulin resistance type-2 diabetes mellitus.

Keywords: *Insulin resistance, dexamethasone, high fat-high sugar, betazen*

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INTRODUCTION

Insulin resistance (IR) is a pathological condition characterised by lack of physiological response from peripheral tissues, skeletal muscle, liver and adipose tissue towards insulin, leading to metabolic and hemodynamic disturbances known as the metabolic disorder [1–4]. IR is a cardinal feature of type-2 diabetes mellitus (T2DM) and is been associated with hypertension, dyslipidemia, and obesity [5]. It mainly occurs due to defects in the insulin signalling cascade and GLUT-4 expression and function in the body [6]. IR involves a complex mechanism wherein there is an excess accumulation of lipids in the muscle and liver. Hence, in insulin-resistant people, normal levels of insulin have an altered effect on muscles, liver and adipose cells. This gives rise to glucose levels, which are higher than the normal level in the body [7]. In addition, excess abdominal fat deposition in obese individuals is been found to be resistant to the anti-lipolytic effects of insulin, thereby leading to the release of excessive amounts of free fatty acids (FFA) and predisposing to ectopic fat accumulation, followed by IR in the liver and muscle. This ultimately contributes to an increase in gluconeogenesis in the liver and an inhibition of insulin-mediated glucose uptake in the muscles resulting in increased levels of circulating glucose [8]. Furthermore, prolonged glucocorticoid therapy as well as high fat and fructose enriched diets are known to induce hyperglycemia, lipolysis, hypertriglyceridemia, oxidative stress and hence, IR in various organs of the body [9–14].

WHO projects that, diabetes deaths will double between 2005 and 2030. It also predicts that diabetes will be the seventh leading cause of death by 2030 [15].

Treatment for people with IR includes advice on nutrition, physical activity, weight loss, smoking cessation and drug therapy. Although insulin therapy and oral hypoglycemics is the main stay of treatment for IR, they have prominent side effects like insulin allergy, resistance, lipoatropy, insulin edema, nausea and vomiting, plasma volume expansion, myalgia, risk of fractures, fluid retention, cholestatic jaundice, agranulocytosis, generalized hypersensitivity reactions, rashes, hypoglycaemia respectively [8,16]. The prophylactic and therapeutic effect of many herbal plant extracts such as *Zingiber officinale* [17], *Momordica charantia* [18], *Trigonella foenum-graecum L* [19], *Azadirachtaindica, Gymnema sylvestre, Picrorhiza kurroa, Pterocarpus marsupium, Tinospora cordifolia*, have been reported for the treatment of IR [20]. In addition, various chemical constituents have been reported to have anti-diabetic property viz.; flavonoids, phenols, sterols, saponins, tannins, glycosides and cinnamaldehyde [21].

As the knowledge of heterogeneity of IR increases, it necessitates the search for more efficacious agents with minimal side effects. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulphonylureas, and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has the capability to modify the course of diabetic complications. However, herbal formulations have shown fewer side effects but lack scientific evidence regarding their safety and efficacy.

Betazen capsules contain a combination of some of the known herbal plant extracts, which include *Curcuma Longa, Melia Azadirachta, Pterocarpus Marsupium, Tinospora Cordifolia, Phyllantus Embilica, Terminalia Chebula, Terminalia Belerica, Zingiber Officinale, Piper Nigrum, Piper Longum, Butea Frondosa, Picrorhiza Kurroa, Gymnema Sylvestre, Trigonella Foenum-Graecum, and Momordicia Charantia* respectively. The herbal formulation 'Betazen' has been widely prescribed by ayurvedic practitioners for insulin resistant diabetes. Despite their use, there is no scientific evidence for its potential activity.

Hence, the present study is been designed to evaluate anti-diabetic activity of betazen herbal formulation on drug and diet induced IR in rats.

MATERIALS AND METHODS

Source of Herbal Formulation

The crude powder contained in 'Betazen' capsules, required for the experimental study was supplied by Progen Research Lab, Belagavi.

Selection of the Dose

The formulation contains various herbal plant extracts for which LD_{50} studies have been reported safe for doses up to 3000 mg/kg. Therefore, the human dose (500 mg) was been converted to animal dose (500×0.018) and each animal was administered with 9 mg/200 g rat dose for the present study.

Experimental Animals

Thirty Wistar rats of either sex weighing 150–180 g, were procured from Shri. Venkateshwara Enterprises, Bangalore. The animals were housed in solid bottom polypropylene cages with a stainless steel grill on top and a bedding of clean paddy husk, at an ambient temperature and humidity, with 12–12 h light and dark cycle. The rats were provided with normal feed and water *ad libitum* for acclimatization to laboratory conditions for a period of 10 days and further provided with the experimental diet. The experimental protocol was approved by the institutional animal ethics committee (Reg. no. 221/CPCSEA), Belagavi.

Experimental Model

For the induction of experimental insulin resistance in rats, the methodology proposed by Renuka *et al.* was been modified slightly and utilized [22]. Wistar rats were rendered insulin resistant by administration of dexamethasone (2 µg/day s.c) for a period of 10 days along with vanaspati ghee and

powdered normal rat feed in the ratio of 2:1 and 25% fructose (HFHS diet) for 42 days. Vanaspati ghee and powdered normal rat feed (2:1) were molded into laddo's and fed to experimental rats (Group II–V) instead of normal pellets while the drinking water was substituted with 25% fructose solution.

Preparation of Drugs

Pioglitazone (PIO) API (active pharmaceutical ingredient), Dexamethasone API and Betazen herbal formulation (BHF) was obtained as a gift sample from Aurobindo Pharmaceuticals (Hyderabad), Centaur Pharmaceuticals (Goa) and Progen Research Lab (Belagavi) respectively. Dexamethasone (2 µg/day s.c) in saline was administered to experimental rats; while, PIO and BHF were separately suspended in 1% CMC solution and administered p.o. to experimental animals.

Experimental Design

Thirty Wistar rats were divided into five groups (six animals/group). Group I served as the normal control and received normal rat feed and potable water. Group II served as the IR group and received dexamethasone (2 µg/day s.c), along with vanaspati ghee and fructose enriched HFHS diet for 42 days. Fasting blood glucose levels (BGL) of all animals were checked weekly and accordingly the insulin resistant rats of group III, IV and V were separately treated with 2.7 mg/kg Pioglitazone, 45 mg/kg, and 90 mg/kg BHF respectively for 30 days.

Parameters Assessed

Body Weight

The body weight of all the animals was recorded initially, at the start of the study and further, weekly during the entire study period. The changes in body weight were monitored.

Blood Glucose Level

Blood samples were collected from overnight fasted rat caudal vein, and estimation of blood glucose levels was performed with the help of on call plus glucometer and glucose strips. The readings obtained were recorded in mg/dl units.

Biochemical Analysis

At the end of the experimental period, rats were anaesthetized and blood samples were collected via retro orbital puncture. The serum was used for the estimation of biochemical parameters viz.; TC, TG, HDL, LDL, VLDL. Whole blood was utilized for HbA_{1C} estimation. The biochemical estimations were been performed using Erba cholesterol kit, Yucca triglyceride kit, Reckon HDL kit and Asritha Diatech HbA_{1C} kit respectively. LDL and VLDL were calculated using Friedewald's formula (Figure 2).

Estimation of Glycogen Content in Liver and Gastrocnemius Muscle of Rat

Glycogen content was estimated by the method described by Caroll, Vies, *et al.* [23, 24] (Figure 3).

Measurement of Glucose Uptake in Hemidiaphragm of Rat

Glucose uptake in rat hemidiaphragm was been estimated by the method described by Chattopadhyay *et al.* with some modification. The glucose content of the incubated medium was measured by GOD/POD, enzymatic method using Erba glucose kit [25].

Estimation of Hepatic Antioxidant Enzymes

Liver homogenates (10% w/v) were prepared in cold 50 mM Tris buffer (pH 7.4). The unwanted cell debris was separated by centrifugation. Further, the supernatant obtained was utilized for the estimation of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and malondialdehyde (MDA) concentration [26–29].

Histopathology

The pancreas were dissected and fixed overnight in 10% formalin. Sections of the tissues fixed in paraffin were prepared, stained with hematoxylin, eosin and observed for pathological changes (Figure 4).

Statistical Analysis

Results are expressed as mean ±SEM. Data was analyzed using one way ANOVA, followed by Dunnett's multiple comparison test (Graph Pad Prism software, version 5.01). $p<0.05$ was considered statistically significant.

RESULTS

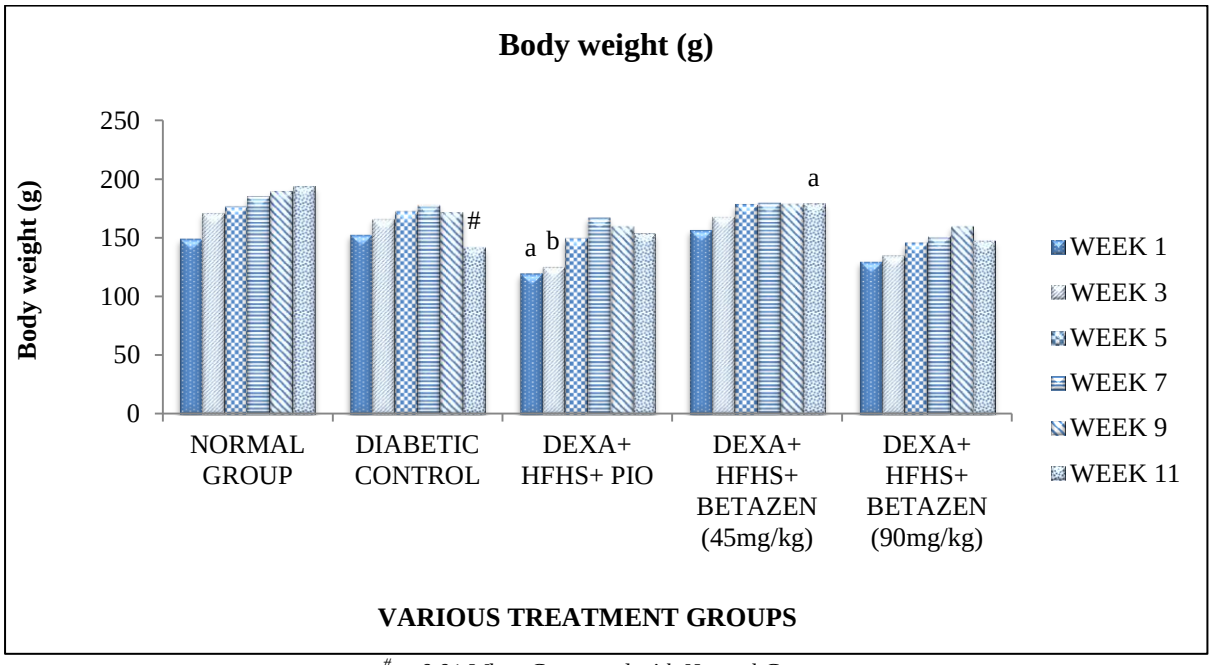
Dexamethasone and HFHS treated IR rats exhibited a significant increase in BGL, VLDL, HbA_{1c} and MDA ($p<0.0001$), total cholesterol and LDL ($p<0.01$) and triglyceride level ($p<0.05$) respectively. In addition, there was a significant ($p<0.0001$) decrease in glycogen content, HDL cholesterol,

antioxidant biomarker's (SOD, GSH and CAT) as well as insulin and non-insulin assisted glucose uptake ($p<0.01$ and $p<0.05$) when compared to normal euglycemic group. Treatment of IR rats with BHF at 45 and 90 mg/kg showed normalisation of all these parameters significantly, in a dose dependant manner (Table 1).

Table 1: *Effect of Various Treatments on Fasting Blood Glucose Levels.*

Week / Groups	Week 1 (mg/dl)	Week 3 (mg/dl)	Week 5 (mg/dl)	Week 7 (mg/dl)	Week 9 (mg/dl)	Week 11 (mg/dl)
Normal Group	81.83±4.586	86.5±3.722	88±2.921	88.67±3.667	89.5±3.51	95.83±4.393
Diabetic Control	96.5±1.784$^{\#}$	100.3±2.512$^{\#}$	109±4.74$^{\#\#}$	140±4.115$^{\#\#\#}$	164±7.75$^{\#\#\#}$	187.8±11.34$^{\#\#\#}$
Dexa+HFHS+PIO	85.17±6.226	91.67±5.499	116.7±7.727	161.8±3.135***	131.2±7.153**	94±8.351***
DEXA+HFHS+Betazen (45 mg/kg)	87.17±1.74	98.33±2.29	135.3±4.333**	162.5±1.803***	147.7±3.04	140.2±5.474**
DEXA+HFHS+Betazen (90 mg/kg)	86.33±.273	106±2.338	139.2±0.8724**	159.3±1.994***	128.2±2.6***	86.5±7.14***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ When Compared with Normal Group.
$^{**}p<0.01$, $^{***}p<0.0001$ When Compared with Diabetic Control.



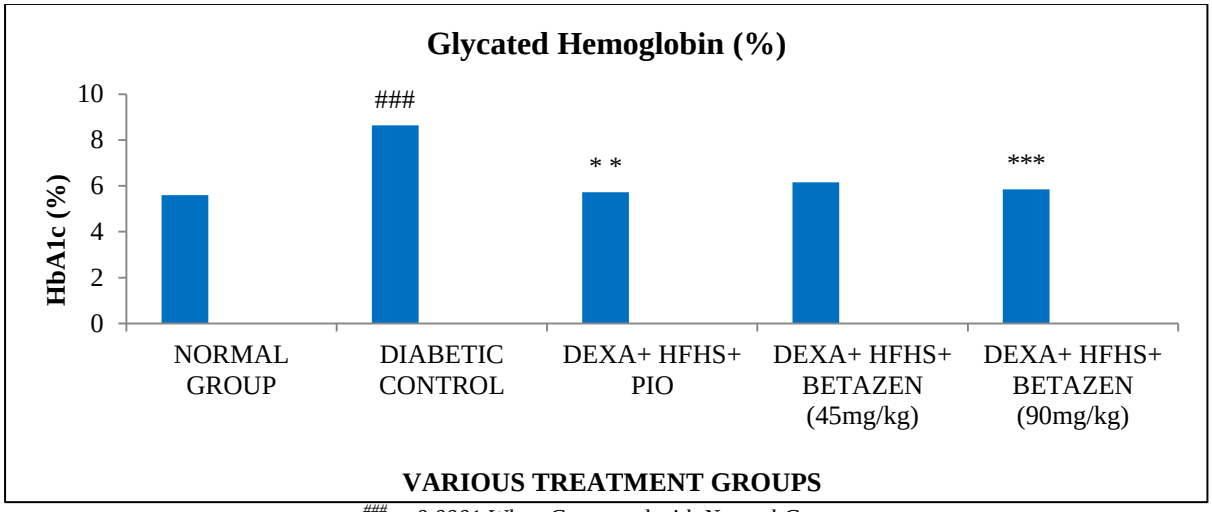
$^{\#}p<0.01$ When Compared with Normal Group.
$^{a}p<0.05$, $^{b}p<0.01$ When Compared with Diabetic Control.

Fig. 1: *Effect of Various Treatments on Rat Body Weight.*

Table 2: *Effect of Various Treatments on Lipid Profile.*

Parameter / Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Group	38.85±2.585	99.51±20.05	61.87±0.845	22.8±2.72	18.97±1.725
Diabetic Control	102.6±13.93$^{\#\#}$	188.5±26.76$^{\#}$	30.83±1.352$^{\#\#\#}$	45.23±4.503$^{\#\#}$	42.61±3.421$^{\#\#\#}$
DEXA+HFHS+PIO	58.5±7.59*	65.88±8.889***	60.12±1.603***	25.58±2.037***	20.88±1.558***
DEXA+HFHS+BETAZEN (45 mg/kg)	66.92±7.538	132.6±12.25	52.59±3.243***	31.98±1.107**	25.13±2.021***
DEXA+HFHS+BETAZEN (90 mg/kg)	78.83±11.07	94.93±7.713**	57.33±2.832***	27.85±2.569***	22.9±1.389***

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.0001$ When Compared with Normal Group.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$ When Compared with Diabetic Control.



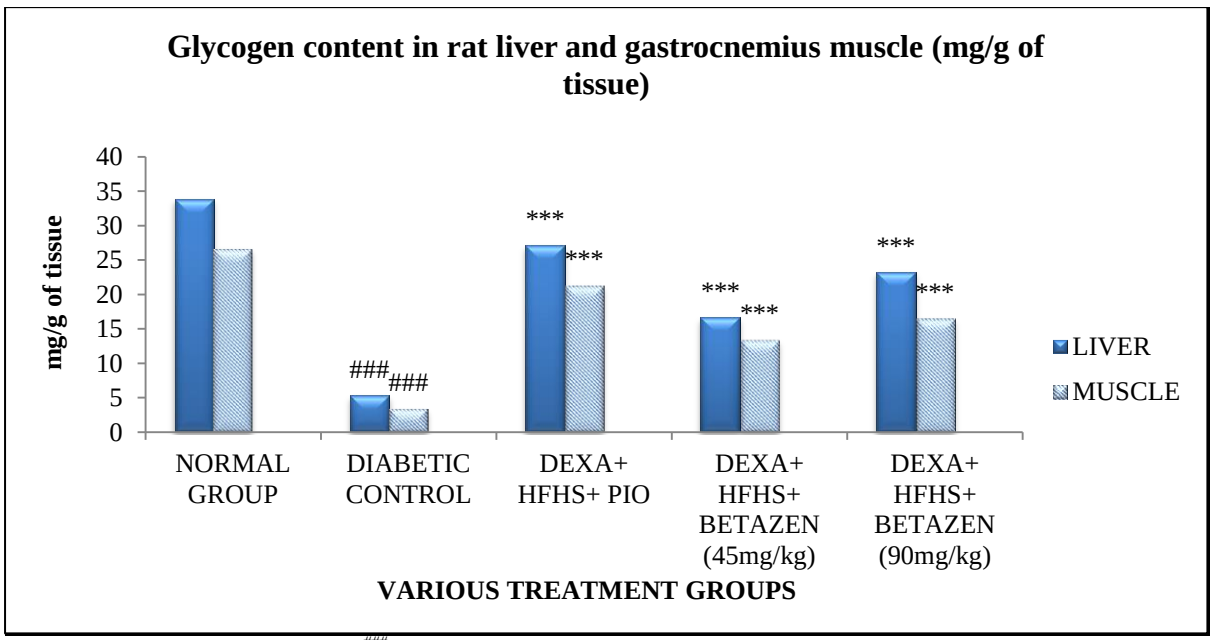
###p<0.0001 When Compared with Normal Group.
***p<0.01, ***p<0.0001 When Compared with Diabetic Control.*

Fig. 2: *Effect of Various Treatments on HbA_{1c}.*

Table 3: *Effect of Various Treatments on Hepatic Anti-Oxidant Enzymes.*

Parameters / Groups	GSH (µg/g of tissue)	MDA (µM/g of tissue)	SOD (U/ml)	CATALASE (U/mg protein)
Normal Group	29.07±1.627	14.44±0.8316	76.55±2.318	19.45±1.88
Diabetic Control	17.46±0.8524###	23.32±1.25###	31.48±1.936###	6.885±0.6632###
DEXA+HFHS+PIO	24.64±1.867**	16.16±0.9175***	73.91±1.878***	17.91±1.583***
DEXA+HFHS+BETAZEN (45 mg/kg)	21.92±1.453	19.5±0.4366*	49.95±4.123***	15.31±0.6934***
DEXA+HFHS+BETAZEN (90 mg/kg)	24.41±1.322**	18.02±0.56***	71.51±2.123***	16.52±1.095***

###p<0.0001 When Compared with Normal Group.
***p<0.01, ***p<0.0001 When Compared with Diabetic Control.*



###p<0.0001 When Compared with Normal Group.
****p<0.0001 When Compared with Diabetic Control.*

Fig. 3: *Effect of Various Treatments on Glycogen Content in Liver and Gastrocnemius Muscle.*

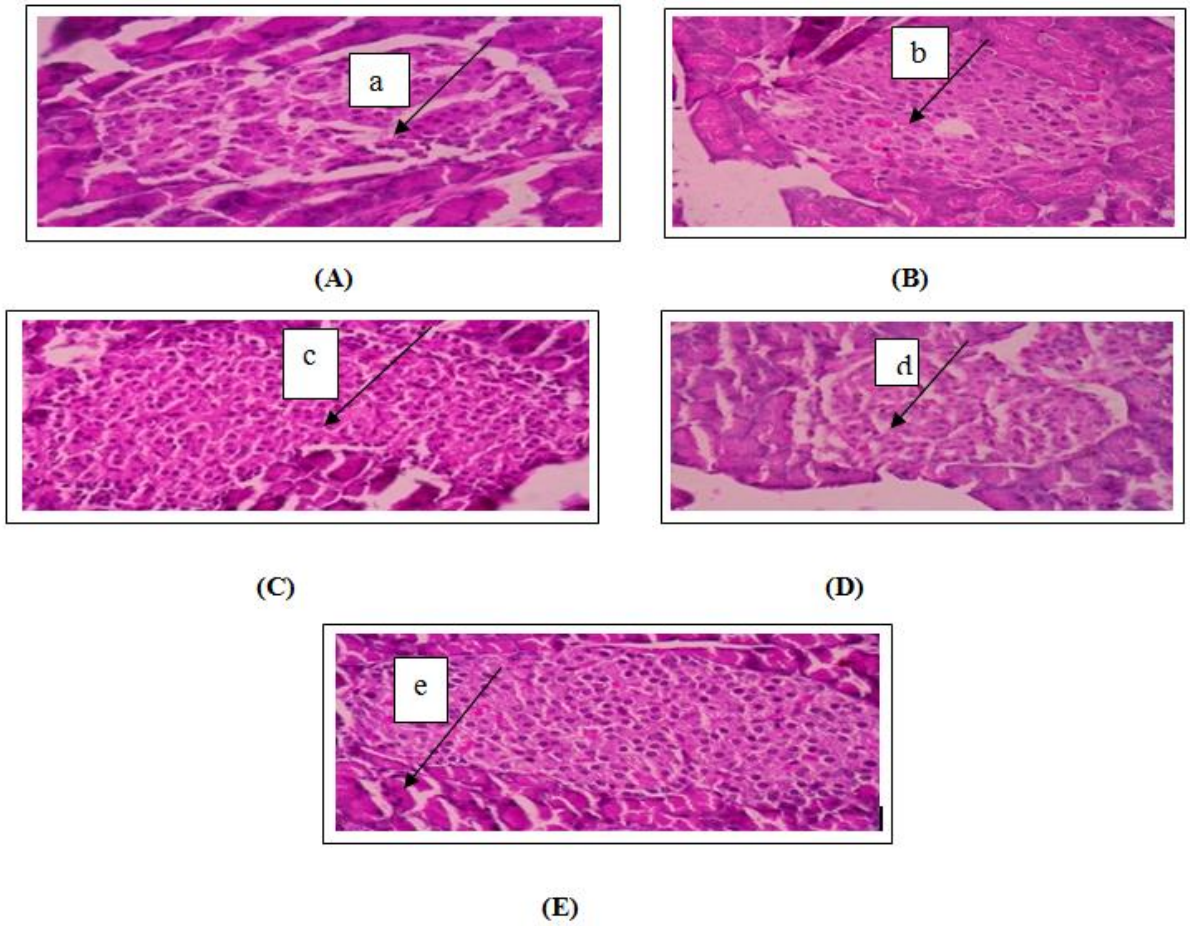


Fig. 4: *Effect of Various Treatments on Histopathology of Pancreas.*
(A) *Normal Group: Section of pancreas showing normal architecture (a).*
(B) *Diabetic Control: Section of pancreas showing moderate decrease in size and number of islets, lymphocytic infiltration and vacuolar degeneration of islet cells (b).*
(C) *DEXA+HFHS+PIO: Section of pancreas showing mild decrease in size and number of islets (c).*
(D) *DEXA+HFHS+BETAZEN (45 mg/kg): Section of pancreas showing mild lymphocytic infiltration, vacuolar degeneration as well as an increase in the number and size of islets (d).*
(E) *DEXA+HFHS+BETAZEN (90 mg/kg): Section of pancreas showing mild lymphocytic infiltration, and increase in the number and size of islets (e).*

DISCUSSION

The present study was designed to evaluate the anti-diabetic effect of betazen capsules, in insulin resistant rats. The doses selected for the present study were 45 and 90 mg/kg respectively. The results obtained from the present study, suggest that betazen herbal formulation (BHF) improves the insulin resistant state in a dose dependent manner. Further, phytochemical investigation of the aqueous extract of betazen capsules has revealed the presence of sterols, flavonoids, alkaloids, tannins, vitamin-C and saponins. Wistar rats were rendered insulin resistant via the administration of dexamethasone (2 µg/day s.c), vanaspati ghee and fructose enriched HFHS diet as a substituent for normal rat feed and potable water respectively. Scientific studies have put forth that dexamethasone, increases glucose levels leading to hyperglycemia. Furthermore, prolonged GC therapy is found to trigger muscle proteolysis, lipolysis, FFA production, steatosis as well as alter IRS-1 transcription in the liver. In addition, GC also prolongs bone resorption and induces oxidative stress in the pancreatic mitochondria, bone and tendon respectively [9–12]. Numerous studies have proved that a high fat diet facilitates the development of obesity. Fat accumulation in adipocytes, muscle and liver cells cause suppression of insulin receptor synthesis, organ dysfunction

and hence, promote an impaired regulation of insulin, blood sugar, cholesterol and heart functions, ultimately leading to IR in these organs. Accumulating evidences have proposed that a fructose enriched diet can induce IR associated with hyperglycemia, hypertriglyceridemia, fatty liver and hypertension by depressing activation of the insulin signalling cascade in the main target tissues viz.; skeletal muscle, liver, and adipose tissue [13, 14].

Results obtained from the present study, demonstrate that administration of dexamethasone and a HFHS diet, induce IR in experimental rats. The study results exhibited a significant increase in BGL, HbA_{1c}, TC, TG, LDL, VLDL, and MDA levels respectively in dexamethasone and HFHS treated insulin resistant rats. In addition, there was a significant decrease in glycogen content, HDL level, antioxidant biomarker's (SOD, GSH and CAT) as well as insulin and non-insulin assisted glucose uptake, thereby highlighting the successful induction of IR.

According to the present study, treatment of IR rats with BHF at 45 and 90 mg/kg showed significant dose dependent reduction in elevated BGL. The observed anti-hyperglycemic effect in the IR rats can be attributed to the phytochemical constituents viz.; flavonoids [30–32], saponins, alkaloids, triterpinoids, phenolics [7, 20, 21], and tannins [33, 34] which have been reported for their anti-diabetic potential in animal experimental studies. These phyto-constituents are been reported to exhibit their anti-hyperglycemic potential via multiple mechanisms viz.; reactivating glycogen synthase enzyme, enhancing pancreatic β-cell function, area, size and number, inhibiting glucose absorption from the gut, improving insulin sensitivity and stimulating insulin mediated glucose transport [7, 20, 21].

In the present study, an initial gradual increase followed by a significant decrease in body weight (BW) was been observed in the IR rats when compared to normal euglycemic group (Figure 1). This decrease in BW is been assumed to occur due to increased muscle wasting or muscle proteolysis mainly because of obesity and dexamethasone administration. Treatment of IR rats with BHF at 90 mg/kg showed a gradual decrease in BW when compared to IR group. Moreover, treatment of IR rats with BHF at 45 mg/kg showed a decrease in BW, followed by maintenance of BW to normal. The maintenance of constant BW can be attributed to the synergistic effects of various contents available in the formulation that due to the presence of mainly flavonoids and alkaloids exhibit thermogenic activity, thus modulating muscle wasting, protein turnover and IR in high fat diet fed rats [35, 36].

Abnormalities in lipid profile are one of the most common complications in IR. Obesity has been implicated in the prevalence of metabolic syndrome, hypertension, IR and dyslipidemia [37]. Moreover, it is been established that dexamethasone, and a fructose enriched diet increases triglyceride levels, causing dyslipidemia. In addition, GC promotes the expression of lipase in peripheral fat deposits, trigger lipolysis, FFA production, hepatic gluconeogenesis, and steatosis in the liver [9–11] (Table 2).

In the present study, the levels of serum TC, TG, VLDL and LDL were significantly increased as well as marked reduction in serum HDL level was observed in IR rats when compared with euglycemic group. This increase in serum lipid levels may be due to lipolysis and resistance towards the anti-lipolytic action of insulin. Treatment of IR rats with BHF at both doses showed significant dose dependant decrease in elevated serum TC, TG, LDL, VLDL as well as significant increase in HDL cholesterol.

The results clearly indicate that treatment with BHF improves lipid abnormalities, and the most prominent effect was observed at the dose of 90 mg/kg BW. The improvement in dyslipidemia may be due to the presence of phyto-constituents viz.; flavonoids, phenolics and saponins that have been reported to exhibit significant anti-hyperlipidemic and antiperoxidative potential as they significantly reduce plasma cholesterol and triglycerides [30–32]. Curcumin, the most important constituent of curcuma longa has been

implicated in the inhibition of lipolysis. While the constituents present in *Azadirachta indica* possess anti-dyslipidemic potential [7]. In addition, Shreya *et al.* put forth that the alkaloid piperine, exhibits lipid lowering ability. The possible mechanism of this action was stated to be due to its thyrogenic activity, thus regulating apolipoprotein levels and IR in rats. Furthermore, piperine also inhibits lipid and lipoprotein accumulation by significantly modulating the activity of enzymes viz.; Lecithin-cholesterol acyltransferase (LCAT) and Lipoprotein lipase (LPL) which are involved in lipid metabolism [35, 36].

During hyperglycemic condition, the elevated blood glucose binds to haemoglobin and form glycosylated hemoglobin. According to the present study, the IR group exhibited a significant increase in glycosylated hemoglobin and this increase was found to be directly proportional to the fasting blood glucose level. Treatment of IR rats with BHF at 90 mg/kg significantly decreased the elevated HbA_{1c} levels. Moreover, treatment with 90 mg/kg showed better effect than the standard drug (PIO). This decline in HbA_{1c} levels may be due to the presence of flavonoids, tannins and phenolics that are proven to have a protective effect on HbA_{1c} levels in animal experimental models [38, 39].

The content of glycogen in various tissues is a direct reflection of insulin activity, since insulin promotes intracellular glycogen synthesis. Hepatic glycogen reserves are important for whole body glucose homeostasis and are markedly low in rats with hyperglycemia. In the present study, a significant reduction in liver and muscle glycogen was observed in dexamethasone and HFHS diet treated rats when compared to normal euglycemic rats. Treatment of IR rats with BHF at 45 and 90 mg/kg showed significant dose dependent increase in liver and muscle glycogen content. This improvement in glycogen content may be due to the presence of alkaloids, lectin and charantin present in *Momordica charantia*, which has been reported to increase glycogen synthesis mainly in the liver, muscles and fat cells. In addition, the constituents present in *Azadirachta indica* have been reported to possess glycogen synthase activation potential [21, 40]. Skeletal muscle is the primary site for insulin stimulated glucose disposal. In the present study, glucose uptake in insulin and non-insulin assisted isolated rat hemi-diaphragm's exhibited a significant decrease in the dexamethasone and HFHS diet treated rats when compared to normal group. The decrease in diaphragm glucose uptake may be due to the insulin insensitivity exhibited by target organs. Treatment with BHF at 45 and 90 mg/kg showed a significant increase in glycogen content. However, in non-insulin assisted glucose uptake only IR rats treated with BHF at 90 mg/kg showed a significant increase in glucose uptake. The increase in glucose uptake may be due to the flavonoids, alkaloids, phenolics and saponins present in *Momordica charantia, Butea monosperma* and *Zingiber officinale* that have been reported to improve peripheral glucose uptake, utilisation and insulin sensitivity respectively [17, 30–32].

Gordon Klein demonstrated that GC induces oxidative stress in the bone, pancreatic mitochondria and tendon. According to the present study, experimental rats to whom dexamethasone and HFHS diet was administered, exhibited a significant reduction in antioxidant biomarker's viz.; SOD, CAT and GSH, while, a there was a significant elevation in MDA levels. This reduction in antioxidant biomarkers and elevation in MDA level in IR rats can be due to the release of free radicals via auto-oxidation and disturbances in the body's antioxidant defence mechanism to combat them. Treatment of IR rats with BHF at 45 and 90 mg/kg showed significant normalisation of these enzymes.

The improvement in antioxidant levels on treatment with BHF may be because of flavonoids, phenolics, tannins and saponins present in some of its contents viz; *Zingiber officinale* [17], *Butea monosperma* [30–32], *Momordica charantia* [40], *Trigonella foenum-graecum, Terminalia chebula* [34] and *Pterocarpus marsupium* [41] that have been reported to possess antiperoxidative and antioxidant potential in diabetic rats. In addition, Picroside I and II (present in *Picrorhiza kurroa* rhizome) have been reported to exhibit significant anti-oxidant potential. This beneficial anti-oxidant potential has been attributed to its inherent ability of

reducing NADPH-oxidase dependent superoxide generation and blunting the expression of MDA and advanced oxidation protein products in diabetic kidney [20, 21]. Furthermore, *Phyllanthus emblica*, is an excellent source of vitamin C and hence exhibits antioxidant property [42] (Table 3).

Histopathological studies of dexamethasone and HFHS diet treated rat pancreas demonstrated mild pathological changes viz.; moderate decrease in size and number of islets, lymphocytic infiltration and vacuolar degeneration of islet cells, when compared to the normal group having normal cellular integrity, thus indicating that IR is not associated with beta cell damage and toxicity. Treatment of IR rats with BHF significantly regulated these abnormalities. The improvement in pancreatic cell integrity is possibly because of the cucurbitane triterpinoids in *Momordica charantia* (contained in Betazen herbal formulation) that has been proposed to increase pancreatic β-cells area, size and number. In addition, phyto-constituents present in *Butea monosperma* have been claimed to stimulate insulin secretion from remnant or regenerated beta cells [30–32].

The results obtained from the present study indicate that BHF shows significant dose dependent improvement in insulin resistant Wistar rats. Based on the present study, it is difficult to establish the exact mechanism of action for improvement in the insulin sensitivity. However, the effect of Betazen herbal formulation can be assumed to be a synergistic effect of mainly phyto-constituents like flavonoids, saponins, triterpinoids, alkaloids, sterols, tannins and vitamin-C present in its contents which act via a plethora of mechanisms to improve IR.

CONCLUSION

In conclusion, BHF demonstrated a significant dose dependent improvement in insulin sensitivity, dyslipidemia, glucose uptake, and glycogen content. Furthermore, it also exhibited antioxidant, anti-glycation and anti-hyperglycemic potential in animal experimental model. This beneficial anti-diabetic effect of betazen herbal formulation can be attributed to the synergistic effects of phyto-constituents viz.; flavonoids, saponins, triterpinoids, alkaloids, sterols, tannins and vitamin-C present in the contents of the formulation. Hence, betazen herbal formulation could be a promising future herbal treatment for insulin resistance.

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