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Glycaemic Evaluation of Folk Recipe (Medicinal Plants) in Alloxan Induced Diabetic Rabbits

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Research Article

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ABSTRACT

Aim/Background: The present study was planned to evaluate the antidiabetic activity of 'Folk recipe' a combination of traditional medicinal plants in normoglycemic and alloxan-induced diabetes rabbits. The level of antioxidant activity was determined by DPPH in relation to the total phenolic contents.
Methods: Antidiabetic activity of aqueous extract of Folk Recipe (AFR) in 100-300 mg/kg, b.w. doses was determined by estimating blood glucose and serum insulin levels before and 1, 2, 4, 8, 24, 48 and 72 hour post-treatment(s) pintervals in treated rabbits. Total phenolic contents and DPPH-antioxidant activity of AFR were measured in vitro.
Results: AFR showed a dose dependent antidiabetic activity; maximum effect was established with 300 mg/kg, b.w. dose. The extract exerted a high significant ($P<0.001$) hypoglycemic effect in normal and alloxan diabetic rabbits. Extract showed a significant ($P<0.05$) increase in insulin levels and protected completely against alloxan-induced histopathological changes in pancreatic beta-cells of diabetic rabbits. A high antioxidant activity of AFR (5-10 µg/mL) was observed in comparison with L-ascorbic acid (5-10 µg/mL). The doses used did not show any sign of acute toxicity or resulted in any behavioral change.
Conclusion: From this study it may be concluded that the Folk recipe causes a reduction in blood glucose and increasing serum insulin levels may combat due to antioxidant activity by protecting beta-cells. Evaluation agreed with the potential use of Folk recipe as a traditional anti-diabetic tool.

Keywords: Folk recipe, hypoglycemic effect, alloxan-induced diabetes, serum insulin, total phenolic compounds and antioxidant;

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1. INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder which is characterized by alteration in carbohydrate, lipid and protein metabolism causing cardiovascular, nephropathic and neurological complications in humans, remains a public health problem worldwide (Zaman, 2006). It has been shown that pathogenic course of both Type 1 and Type 2 DM involves alterations in the structures, organization and protein functions of membranes of cells and tissues (e.g. retina, glomerulli, erythrocyte, nerve), culminating in diabetic complications such as retinopathy, nephropathy and peripheral neuropathy (Budak et al., 2004; Carneiro, 2004; Zaman, 2006). Hyperglycemia is clinical hallmark of DM but etiology of this heterogeneous disorder likely involves multiple genetic and environmental interactions that ultimately result in alterations in insulin secretion, insulin action or both (American Diabetes Association, 2006). Several approaches like lifestyle changes, food intake modifications; lowering the fat content (Van Dam et al., 2002) or enhancing the fiber and magnesium content of the diet (Lopez-Ridaura et al., 2004) and/or physical activity promoting weight loss (Kosaka et al., 2005), smoking status (Tuomilehto, 2005), moderate coffee (Van Dam and Hu, 2005), moderate alcohol consumption (Conigrave et al., 2001) and finally bariatric gastric surgery (Sjöström et al., 2004) have been tried for the prevention of DM (Gruber et al., 2006). In addition many oral antidiabetic agents have been evaluated in the context of DM prevention (Mancini and Halpern, 2008). The role of blood pressure lowering, diuretics, beta-blockers, calcium channel antagonists has also been studied for the prevention of disease. The benefits of hypolipidemic agents and hormone replacement therapy in DM prevention have been assessed (Mancini and Halpern, 2008).

Most antidiabetic drugs are hypoglycemic or anti-hyperglycemic. However, most of these drugs are adipogenic (Moller, 2001). Thus, these drugs treat one of the key symptoms of type 2 diabetes, hyperglycemia, but exacerbate the condition of being overweight or obese, one of the leading causes of type 2 diabetes. Therefore, while these drugs are beneficial over the short term; they are not optimal for long term health of type 2 diabetic patient. Currently-available drug regimens for management of *Diabetes mellitus* have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drug(s) (Kaleem et al., 2008).

The pharmaceutical drugs are either too expensive or have undesirable side effects. Treatment with Sulphonylureas and Biguanides are also associated with side effects (Kaleem et al., 2006). Most desirable situation would be the development of new types of antidiabetic drugs that are either hypoglycemic or anti-hyperglycemic without the side effects and of promoting weight gain (adiposity). Herbal medicines known to be useful in diabetes treatment may be able to lead to compound(s) with such a combination of ideal therapeutic properties (Samane et al., 2006; Iwalokun et al., 2007).

Medicinal plants have long been used for the treatment of DM. The disease was continued to manage entirely with such indigenous plants until the development of insulin injection therapy in 1921 (Wadood et al., 2002). Several such plants have been identified hypoglycemic either individually or in combinations (Sabu and Kuttan, 2002; Huo et al., 2003; Winters et al., 2003; Wadood et al., 2003).

The combination of medicinal plants used during the course of current investigation was folk recipe (FR), a combination of three following medicinal plants.

1. *Citrullus colocynthis* (*Cucurbitaceae*) Fruit
2. *Acacia modesta* Wall (Mimosaceae) Bark
3. *Polygonum fagopyrum* L (Polygonaceae) Seed

The fruits of *Citrullus colocynthis,* commonly known as bitter apple, are bitter, acrid, cooling, cathartic, carminative, antipyretic, anthelmintic and are useful in hypoglycemia, tumors, ascites, leucoderma, ulcers, asthma, bronchitis, urethorrhea, jaundice, dyspepsia, constipation, elephantiasis and splenomegaly (Marzouk et al., 2009). Fruit extract exhibits nematicidal properties (Kumar et al., 2008). *Acacia modesta* is used as a tonic. Both gum and bark are traditionally used as anti-diabetic (Ullah et al., 2010). Singh et al. (1975) have been reported the analgesic, anti-inflammatory and anti-platelet activities of *Acacia modesta* leguminous seed diets. Locally it is used as miswak (chewing stick) in various parts of Pakistan due to its effectiveness against dental diseases and gastric disorders (Asghar et al., 2003). Akhtar et al., (1997) have reported antibacterial activity of *Acacia modesta.* Leave, fruit, bark and wood is also used commonly for medicinal, fuel and timber purposes (Asghar et al., 2003). *Polygonum fagopyrum* L. is a crop for food as well as medicine. Seeds are widely used as health food. Whole plant is rich in flavonoids and rutin, which exists mostly in the flowers especially flower buds followed by leaves (Shu-Ying et al., 2010).

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL AND EXTRACT

Citrullus colocynthis fruit, *Acacia modesta* Wall bark and *Polygonum fagopyrum* L. seed (Folk Recipe) were purchased from the local herbal dealer at Bahawalpur-Pakistan. The plant materials were authenticated and compared with their standards in the herbarium maintained by Department of Pharmacy, the Islamia University of Bahawalpur-Pakistan. Separate specimens of these plant drugs (FR. Ph. 101-3) were preserved in the Pharmacognosy laboratory, Department of Pharmacy, the Islamia University of Bahawalpur-Pakistan (Zaman and Rehman, 2010).

Dried, ground materials (1.0 kg) were macerated with distilled water (about 2.0 Lit each) at room temperature for 24 h separately. Dried extracts obtained were stored in the sealed container at 4^{o}C before use in experiment (Zaman et al., 2004). The aqueous extracts were mixed in equal weights (equivalent to respective plant drug) before their administration for test.

2.2 ANIMALS

Adult healthy male rabbits (*Oryetolagus cuniculus*) of local bread, weighing about 1.20-1.50 kg were used in this experiment. Animals were acclimatized under the standard conditions of temperature (23±12^{o}C), humidity (55±15%) and 12 h light (7.00-19.00). Rabbits were kept under observation for a week in animal house of Department of Pharmacy, the Islamia University of Bahawalpur before using in the experiment. Animals were provided with a free access to a balanced rabbit's diet consisting of green leaves, fodder, pulses (*Medicago sativa*) and water *ad libitum*. They were fed according to a strict schedule (6.00, 14.00 and 20.00 h). Overnight fasted animals were divided randomly into different groups (6-8 animals

per group) that were used in accordance with the NIH guide for the care and use of laboratory animals in this study (Zaman and Ahmad, 2004).

2.3 DIABETES INDUCTION

Diabetes mellitus (DM) was induced by a single intravenous (*IV*) injection of Alloxan monohydrate (150 mg/kg, b.w.), dissolved in 0.1 M sodium citrate buffer pH 4.5. The control group received similar volume of the vehicle (citrate buffer, 1 ml/kg). In order to reduce death due to hypoglycemic shock, alloxan-treated rabbits received 5% of glucose instead of water for 24 h after diabetes induction (Barbosa et al., 2008).

2.4 CHEMICALS

All chemicals used; Alloxan monohydrate, Sodium carbonate, L-ascorbic acid, 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), Folin-Ciocalteu, Gallic acid, Methanol, were obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA). Glucose estimation kit (Diagnostics Elitech kit) and insulin estimation kit (enzyme-linked immuno sorbent assay kit) were obtained by ELISA, Boerhringer Mannheim, Germany. The reference diabetic drug, Metformin was taken from Ferozsons Laboratories Limited, Rawalpindi, Pakistan.

2.5 ADMINISTRATION OF DRUGS

On experimental day (01) three groups of fasted-normal and three groups of fasted-treated rabbits were administered either 100, 200 or 300 mg/kg, b.w. (Rai et al., 2007) of aqueous extract of Folk Recipe (AFR) while fasted-reference control rabbits were given Metformin (500-mg/kg, b.w.) (Aritajat et al., 2004) and fasted-untreated control (normal) rabbits were given lactose (500-mg/kg b. w.) in gelatin capsule 12 h for three consecutive days (1-3) orally. One hour later the treated, treated-control and reference-control rabbits were injected with Alloxan monohydrate ($C_4H_2N_2O_4.H_2O$, 10%) 150 mg/kg, b. w. intravenously (*IV*) while an equal volume of vehicle (*IV*) to all other rabbits (Zaman and Rehman, 2010).

2.6 LABORATORY ANALYSES

Blood samples from all the rabbits under test were collected before and after 1, 2, 4, 8, 24, 48 and 72 h intervals following the Alloxan injection. Specimens were centrifuged at 6000 rpm for 15 min. Serum was used for the estimation of glucose by the Diagnostics Elitech kit method (GLUCOSE HK SL-reference # GHSL-0600). Serum insulin level was assayed by enzyme-linked immuno sorbent assay kit method (ELISA method: Enzyme Linked Immunosorbent Assay; ELISA, Boerhringer Mannheim, Germany, Cat. No. KT-438) (Kaleem et al., 2006).

2.7 HISTOPATHOLOGICAL EXAMINATION

On experimental day 3 the animals were sacrificed and their pancreas were removed and fixed in 10% formalin and processed for paraffin embedding. Tissues were sliced 6 mm in thickness with microtome. Slices were embedded in paraffin wax and sectioned at 5 µm. Sections were stained with haematoxylin, eosin and mounted in Canada balsam. Histopathological assessment was done according to the standard method (Garba et al., 2009).

2.8 ANTI-OXIDANT ASSAY

2.8.1 Total phenolic compound (TPC) measurement

Total amount of phenolic compounds (TPC) in ethanol, chloroform, ethyl acetate and aqueous extracts of Folk Recipe (AFR) was determined by Folin-Ciocalteu reagent method (Djeridane et al., 2006). 0.4 mL Folin-Ciocalteu (1:10) solution was shaken thoroughly with 50 µL of AFR (20 µg/mL) solution. Mixture was allowed to stand for 30 min after addition of 0.8 mL of sodium carbonate, 7.5% solution with occasional shaking. Absorbance of test solution was measured spectrophotomerically at 746 and TPC was expressed as Gallic acid equivalent (mg/g of extract) from calibration cure of Gallic acid standard solution (Changwei et al., 2008).

2.8.2 DPPH anti-oxidative activity determination

Free radical scavenging activity was determined by partialy modified method of Gyamfi et al. (1999). Test substance at different concentration (2.5, 5.0 and 10 µg/mL of AFR) dissolved in methanol, which was added to 1.5 x 10^{-4} M 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) methanol solution. The solution with the substance was shaken vigorously and kept in dark for 30 min at 25^{o}C. The absorbance (A) of the test solution was measured spectrophotomerically at 520 nm. L-ascorbic acid was used as a positive control (2.5-10 µg) (Jeong, 2002).

The percentage inhibition values were calculated by using following equation (Lompo et al., 2007; Millogo-Kone et al., 2009):

$$\text{DPPH scavenging (\%)} = (A_{control} - A_{test} / A_{control}) \times 100$$

2.9 STATISTICAL ANALYSIS

Mean values of six experiments ± S.E.M (standard error of means) were analyzed for statistical significance by one-way ANOVA test. Results were considered significant at $p<0.05$ (Zaman, 2006).

3. RESULTS AND DISCUSSION

Aqueous extract of *Folk Recipe* (AFR) exhibited a dose-dependent hypoglycemic effect in the normoglycemic (fasted-normal) rabbits in the present study (Figure 1). 300 mg/kg, b.w. dose caused the most potent effect which was started with the administration of AFR ($P<0.05$ at 1 h post-treatment) and reached maximum ($P<0.001$) at 2-6 h intervals. A significant hypoglycemic effect ($P<0.05$) in comparison to the fasted-control rabbits was observed at 24 and 48 h test points (Figure 1). Other test doses of AFR showed similar but less potent hypoglycemic effect in descending order (200→100 mg/kg, b.w.); 200 mg/kg dose decreased blood glucose level significantly at 1, 2 h while this decrease was high significant at 4, 6 h intervals. However 100 mg/kg dose caused hypoglycemia, significantly at 2 h and high significantly at 4, 6 h intervals (Figure 1). The finding is in consistent to Sabu and Kuttan, (2002); Syiem et al., (2002); Wadood et al., (2003).

A single dose of alloxan (150 mg/kg, b.w.) induced a highly significantly ($P<0.001$) elevated blood glucose level at 2-72 h intervals in the fasted-treated control rabbits and maximum

hyperglycemic effect (P=0.000005) was observed at 6 h post-treatment (Figure 2, Table 1). Sabu and Kuttan (2002) and Wadood et al. (2003) found similar effects in alloxan diabetic rabbits. The alloxan-induced hyperglycemic effect in the fasted-treated rabbits was attenuated successfully by AFR in a dose-dependent manner (Figure 2). 300 mg/kg, b.w. dose ameliorated this effect significantly (P<0.001) followed by 200 and 100 mg/kg, b.w. doses. Complete antagonism (P=0.001842-0.000001 at 1-72 h test points) by 300 mg/kg dose was observed in treated rabbits (Figure 2, Table 1). Grover et al. (2002) and Wadood et al. (2003) reported such activity of various indigenous medicinal plants. Metformin (500-mg/kg, b.w.), a reference agent antagonized successfully alloxan-induced hyperglycemia, however this effect was short-lived which was abolished gradually (P=0.000927 at 6 h, P=0.347914 at 24 h and P=0.492067 at 48 h test points) (Table 1). The finding is comparable with Syiem et al. (2002); Shan et al. (2006); Nabeel et al. (2010).

Alloxan further induced a marked decrease in serum insulin level at 1-72 h intervals in the fasted-treated control rabbits and maximum hypoinsulinemia (P=0.001433) was observed at 4 h post-treatment (Table 2). AFR inhibited dose-dependently, alloxan-induced decline in serum insulin level in fasted-treated rabbits (Table 2). Most potent effect was caused by 300 mg/kg, b.w. dose (P=0.010120-0.003302 at 1-72 h test points) (Table 2). Metformin also antagonized alloxan-induced hypoinsulinemia up to 6 h test point but this effect was less potent in comparison to AFR (300 mg/kg), which is in agreement with the findings of Kanigür-Sultuybek et al. (1995).

A fairly narrow normal range of serum glycemia is regulated by the release of insulin (β-cells) and glucagon (α-cells) from pancreatic islets (Aronoff et al., 2004). In general, insulin is the hormone responsible for glucose disposal, glucagon for glucose availability. Hyperglycemia promotes insulin release, which in turn increases formation of glycogen stores, facilitates glucose uptake in muscle and adipose tissues, and suppresses hepatic glucose output, in part via paracrine suppression of glucagon release. The reduced glucagon secretion limits hepatic glucose output through suppression of glycogenolysis and gluconeogenesis. The opposite series of events occurs during the fasting state, in which the effects of glucagon predominate and insulin secretion is minimal (Green and Feinglos, 2008). Loss of glycemic control due to abnormalities of insulin action, including deficiency and insulin resistance (Cheng et al., 2006) may cause hyperglycemia leading to *Diabetes mellitus* (American Diabetes Association, 2006).

Data of present study indicated, that Alloxan induced, a significant hyperglycemia (P=0.000005) and decreased significantly (P=0.006577) serum level of insulin in fasted-treated control rabbits (Table 1 and 2). AFR (300 mg/kg, b.w.) antagonized completely alloxan-induced effects on serum levels of glucose and insulin in fasted-treated rabbits (Table 1 and 2). Metformin (500 mg/kg, b.w.), a reference antidiabetic agent also protected (P<0.001) alloxan-induced hyperglycemic and hyporinsulinemic effects in fasted-reference control rabbits (Table 1 and 2).

Table 1. Effect of aqueous extract of Folk Recipe (300 mg/kg, b.w.) on serum glucose levels at different time intervals in Rabbits

Time interval (Hours)	Blood glucose level (mg/dL)			
	Untreated Control (Normal)	Alloxan (150 mg/kg) (Diabetic) (Treated control)	Alloxan (150 mg/kg) + Folk Recipe (300 mg/kg) (Treated)	Alloxan (150 mg/kg) + Metformin (500 mg/kg) (Reference treated)
0	103.54 ± 3.16	105.42 ± 2.02 (P=0.318723)	103.23 ± 2.06 (P=0.482025)	104.17 ± 3.36 (P=0.381383)
1	101.19 ± 2.54	121.83 ± 2.45 (P=0.001035)	105.15 ± 2.14 (P=0.001842)	115.37 ± 3.85 (P=0.107724)
2	102.19 ± 2.99	187.28 ± 3.45 (P=0.000004)	107.66 ± 2.52 (P=0.000004)	137.49 ± 4.63 (P=0.000173)
4	102.65 ± 2.78	215.14 ± 3.58 (P=0.000001)	108.78 ± 2.65 (P=0.000001)	148.51 ± 4.62 (P=0.000045)
8	99.31 ± 2.87	224.22 ± 4.08 (P=0.000005)	107.76 ± 3.35 (P=0.000004)	180.13 ± 6.12 (P=0.000927)
24	101.67 ± 2.32	221.79 ± 6.38 (P=0.000005)	107.35 ± 4.25 (P=0.000008)	217.41 ± 8.43 (P=0.347914)
48	102.91 ± 2.98	215.14 ± 5.86 (P=0.000006)	105.54 ± 3.76 (P=0.000009)	215.34 ± 7.56 (P=0.492067)
72	104.12 ± 1.79	216.69 ± 6.12 (P=0.000005)	105.97 ± 4.57 (P=0.000009)	216.33 ± 7.72 (P=0.486148)

Treated and Reference treated: compared with treated control, Treated control: compared with untreated control (Normal) at respective time interval, Mean ± S.E.M = Mean values ± Standard error of means of six experiments.

Table 2: Effect of aqueous extract of Folk Recipe (300 mg/kg, b.w.) on serum insulin levels at different time intervals in Rabbits

Time interval (Hours)	Serum Insulin Level (µIU/mL)			
	Untreated Control (Normal)	Alloxan (150 mg/kg) (Diabetic) (Treated control)	Alloxan (150 mg/kg) + Folk Recipe (300 mg/kg) (Treated)	Alloxan (150 mg/kg) + Metformin (500 mg/kg) (Reference treated)
0	10.24 ± 1.54	105.42 ± 2.02 (P=0.318723)	103.23 ± 2.06 (P=0.482025)	104.17 ± 3.36 (P=0.381383)
1	10.93 ± 2.02	121.83 ± 2.45 (P=0.001035)	105.15 ± 2.14 (P=0.001842)	115.37 ± 3.85 (P=0.107724)
2	10.91 ± 1.95	187.28 ± 3.45 (P=0.000004)	107.66 ± 2.52 (P=0.000004)	137.49 ± 4.63 (P=0.000173)
4	11.22 ± 1.26	215.14 ± 3.58 (P=0.000001)	108.78 ± 2.65 (P=0.000001)	148.51 ± 4.62 (P=0.000045)
8	11.14 ± 1.88	224.22 ± 4.08 (P=0.000005)	107.76 ± 3.35 (P=0.000004)	180.13 ± 6.12 (P=0.000927)
24	10.16 ± 2.10	221.79 ± 6.38 (P=0.000005)	107.35 ± 4.25 (P=0.000008)	217.41 ± 8.43 (P=0.347914)
48	10.18 ± 1.87	215.14 ± 5.86 (P=0.000006)	105.54 ± 3.76 (P=0.000009)	215.34 ± 7.56 (P=0.492067)
72	10.52 ± 2.18	216.69 ± 6.12 (P=0.000005)	105.97 ± 4.57 (P=0.000009)	216.33 ± 7.72 (P=0.486148)

Treated and Reference treated: compared with treated control, Treated control: compared with untreated control (Normal) at respective time interval, Mean ± S.E.M = Mean values ± Standard error of means of six experiments.

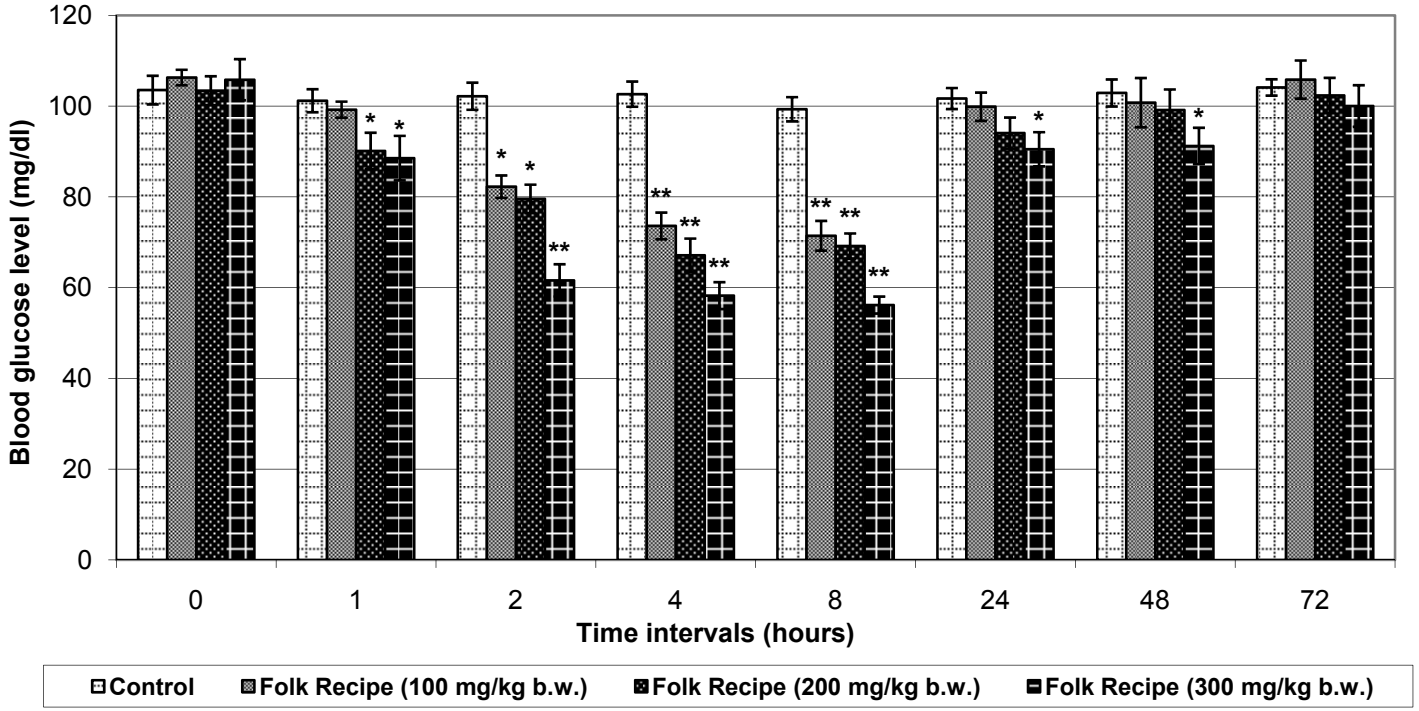

Figure 1. Effect of different doses of aqueous extract of Folk Recipe (100, 200, 300 mg/kg) on blood glucose levels at different time intervals in normal rabbits

*Test drugs: significant from normal control, * P < 0.05; ** P < 0.001*

Mean ± S.E.M = Mean values ± Standard error of means of six experiments

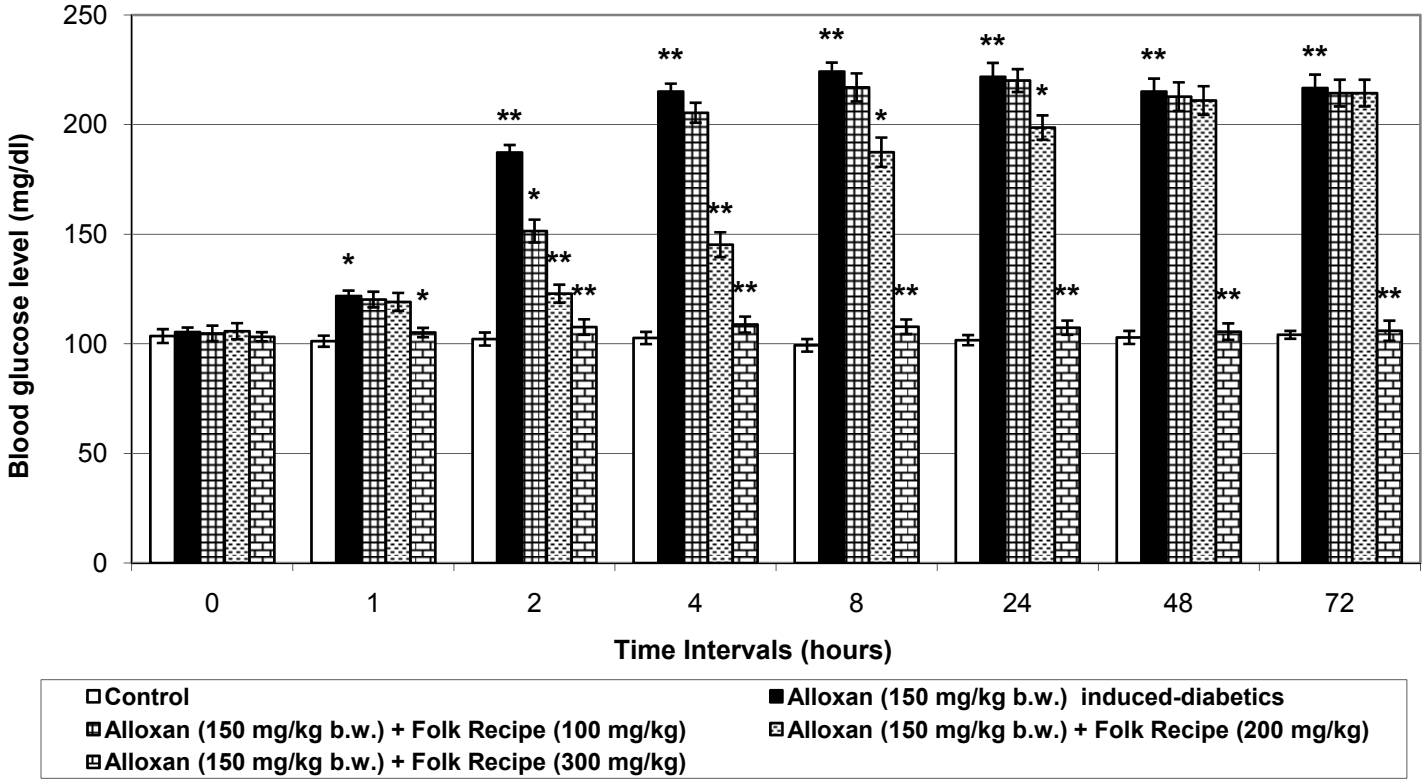

Figure 2. Effect of different doses of aqueous extract of Folk Recipe (100, 200, 300 mg/kg) on blood glucose levels at different time intervals in Alloxan (150 mg/kg, b.w.)-induced diabetic rabbits

*Test drug: significant from treated control (Alloxan), Alloxan: significant from control (Normal) * P < 0.05; ** P < 0.001;*
Mean ± S.E.M = Mean values ± Standard error of means of six experiments

Histological studies of the endocrine region of pancreas of the Alloxan-diabetic rabbits revealed the presence of damaged β-cell population (Figures 3 and 4, Table 3). This reduction in β-cell number was less than 50% during diabetes which is in accord to Hayashida et al., (1983). Alloxan treatment further induced shrinkage and necrosis of β-cell in diabetic rabbits (Figures 3 and 4, Table 3). On the other hand, AFR treated diabetic rabbit revealed restoration of size of the islets along with β-cells repairment. This β-cells recovery was recorded in the dose dependant manner *i.e*. 100→300 mg/kg, b.w. (Figure 5, 6 and 7). AFR in 300 mg/kg, b.w. dose exhibited complete antagonism against alloxan induced β-cell toxicity (Figure 7) while Metformin, a reference drug protected the β-cell partially (Figure 8). Histopathological findings are comparable with the findings of Gohil et al. (2010).

Table 3. Pancreatic histopathological changes induced by aqueous extract of Folk Recipe (AFR) in Alloxan-treated diabetic rabits

Groups	Treatment	Pancreatic β-cells			
		Number	Shrinkage	Necrosis	Repair
1	Normal	N	-	-	N
2	Alloxan 100 mg/kg, *IV*	L	+++	+++	-
3	Alloxan 100 mg/kg, *IV* + AFR 100 mg/kg, *po*	L	++	+	-
4	Alloxan 100 mg/kg, *IV* + AFR 200 mg/kg, *po*	L	+	-	+
5	Alloxan 100 mg/kg, *IV* + AFR 300 mg/kg, *po*	N	-	-	N
6	Alloxan 100 mg/kg, *IV* + Metformin 500 mg/kg, *po*	L	++	+	-

IV; intravenous, N: normal, L: less in number, -: no change, +: mild, ++: moderate, +++: sever, AFR: aqueous extract of Folk Recipe

Alloxan is a β-cytotoxin, induces *Diabetes mellitus* by damaging the insulin secreting β-cells of the pancreas, resulting in decreased endogenous insulin release. Alloxan-administered rabbits become hyperglycemic in a short period of time, followed by hepatic glucose overproduction (Milagro and Martínez, 2000; Rajagopal and Sasikala, 2008). High ambient glucose can promote apoptosis, suggested by Allem et al. (2003), causing potential cellular damage as a result of hyperglycemia in diabetes. Reactive oxygen species (ROS) are important mediators of β-cell death during the development of DM. High glucose has been postulated to generate ROS and nitrogen species in numerous cell types. Generation of superoxide by high glucose is well described and arises principally via the mitochondrial electron transport chain (Chung, et al., 2003). Another source of glucose-induced oxidative stress is via the polyol pathway where glucose is reduced to sorbitol by aldose reductase in a process that consumes NADPH. This will impair the NADPH-dependent generation of glutathione, an essential cellular antioxidant (Du, et al., 1999; Dallak, et al., 2008). Pancreatic islets exhibit greater susceptibility to damage by ROS compared to other tissues as a result of lower antioxidant defenses (Li, et al., 2002; Chen, et al., 2008). Alloxan-induced free radical-mediated diabetes, led to a strain of rabbit with an elevated systemic as well as pancreatic ROS dissipation (Shi and Vanhoutte, 2009).

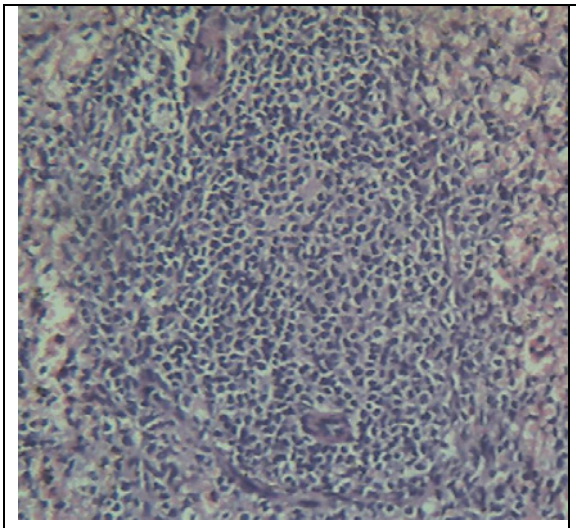

Figure 3: β-cells of pancreatic Islet of Langerhans of Normal Control Rabbit.

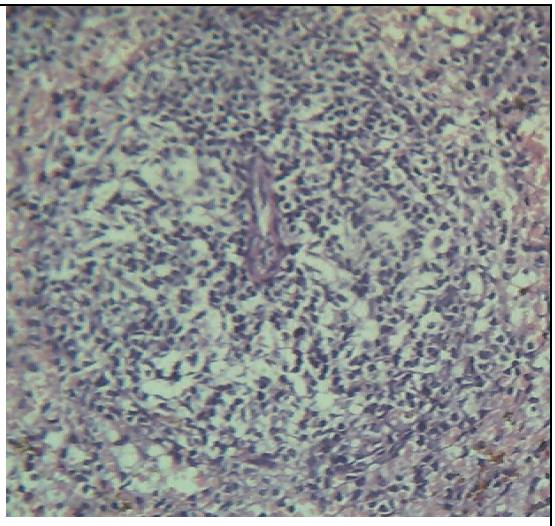

Figure 4: Alloxan (100 mg/kg b. w.) induced destruction in pancreatic β–cells in toxic control rabbits.

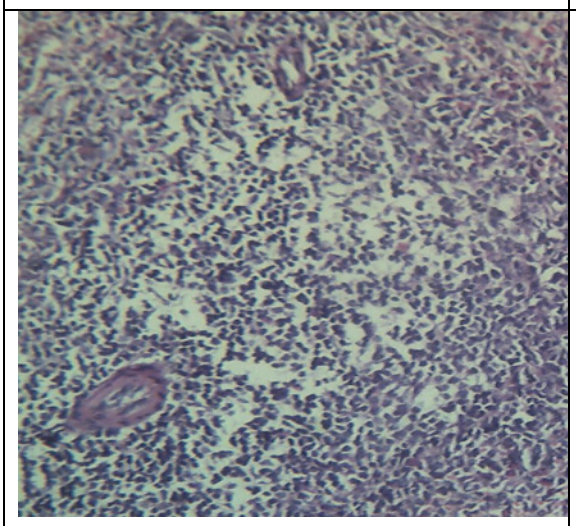

Figure 5: Partial antagonistic effect caused by AFR (100 mg/kg b. w.) in pancreatic β–cells in Alloxan (100 mg/kg) treated rabbits.

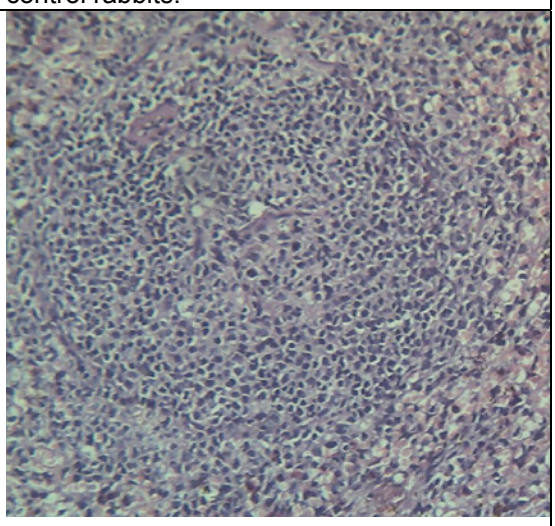

Figure 6: Antagonistic effect in pancreatic β–cells caused by AFR (200 mg/kg) in Alloxan (100 mg/kg) treated rabbits.

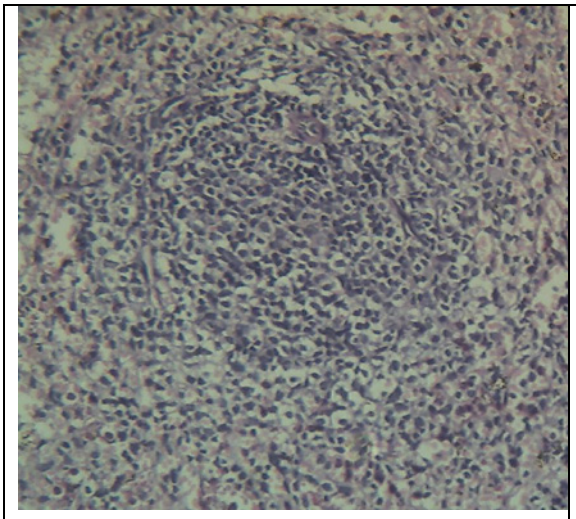

Figure 7: Complete inhibition of Alloxan (100 mg/kg) effect by AFR (300 mg/kg) in pancreatic β–cells of treated rabbits.

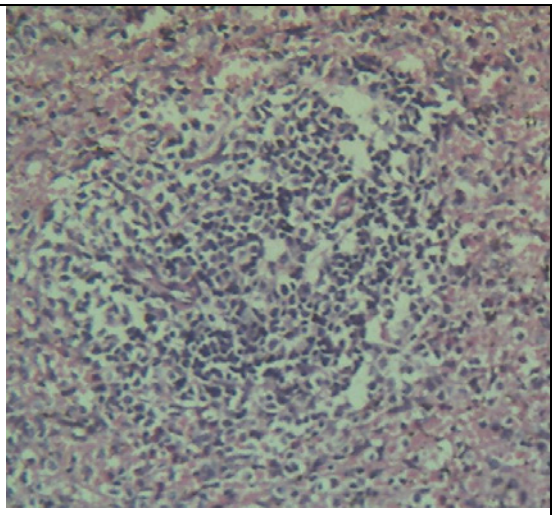

Figure 8: Partial attenuation caused by Metformin (500 mg/kg) in pancreatic β–cells in Alloxan (100 mg/kg) treated rabbits.

Table 4. Antioxidant effect of aqueous extract of Folk Recipe (AFR)

S. #	Treatment(s)	Concentration (µg/mL)	Inhibition (%)	p-value
01	L-ascorbic acid	2.5	8.2 ± 0.75	-
02	L-ascorbic acid	5.0	17.3 ± 1.24	-
03	L-ascorbic acid	10.0	75.9 ± 3.21	-
04	Folk Recipe	2.5	9.4 ± 0.61	P= 0.170182
05	Folk Recipe	5.0	26.3 ± 2.50	P= 0.042092
06	Folk Recipe	10.0	96.4 ± 4.73	P= 0.034863

Folk Recipe: compared with L-ascorbic acid (respective concentration);
Mean ± S.E.M = Mean values ± Standard (n=3)

Table 5. Total phenolic contents of Ethanol, Chloroform, Ethyl acetate and aqueous extracts of Folk Recipe

Folk Recipe Extract in	Total phenolic contents (Gallic acid equivalent, mg/g extract)
Ethanol	3.58 ± 0.61
Chloroform	0.56 ± 0.07
Ethyl acetate	1.83 ± 0.28
Water	5.91 ± 0.36

AFR dose-dependently increased DPPH free radical scavenging activity. Aqueous extract showed significantly high (P=0.034863) activity in comparison with L-ascorbic acid, a positive control at 10 µg/ml concentration (Table 4). The total phenolic contents level was

highly correlated with the free radical scavenging activity (Table 5). This finding is comparable with reports of Heo et al. (2007) and Al-Mustafa & Al-Thunibat (2008).

It may be speculated from above mentioned data that AFR may cause its hypoglycemic, hyper-insulinemic effect in normoglycemic and antidiabetic effect as well as amelioration of histopathological changes in alloxan-induced diabetic rabbits may be due to free radical scavenging activity. The finding is in accord with Gallo et al., (2005); Wadood et al., (2007); Al-Mustafa and Al-Thunibat, (2008). Metformin, an insulin sensitizer (Hundal and Inzucchi, 2003) showed antidiabetic activity because of a direct antioxidant effect mediated by inhibiting NADPH oxidase and stimulating catalase activity (Gallo et al., 2005). The findings further pointed out that AFR showed more potent and prolong antidiabetic activity in comparison to Metformin (Figure 2, Table 1-3).

4. CONCLUSION

The high total phenolic contents of AFR may correlate with the free radical scavenging activity. Test extract may be beneficial to control the diabetes by hypoglycemic effect, increasing insulin levels and protecting pancreatic β-cells may combat due to antioxidant activity. Evaluation agreed with the potential use of *Folk Recipe* as a traditional anti-diabetic tool.

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