Anti-diabetic and hypolipidemic effects of extract from the seed of *Gossypium herbaceum* L. in Alloxan-induced diabetic rabbits

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Abstract: Anti-diabetic and hypolipidemic effects of seed of *Gossypium herbaceum* L (GH) and its aqueous and ethanol extracts were investigated in alloxan-induced diabetic rabbits. Normal, Alloxan-induced diabetic and treated groups of rabbit were examined for their serum glucose, triglyceride, cholesterol, creatinine and urea levels. Water/food intake and toxic effect of test substances were also observed in treated rabbits. Effect of test agents on architecture of pancreatic β-cells was evaluated histopathologically in rabbits. GH powder, its aqueous (GHA) and ethanol (GHE) extract significantly ($P<0.05$) reduced normoglycemia, serum cholesterol, triglyceride and urea in a dose dependent order (200→300 mg/kg of body weight) in normal rabbits. GH and GHE ameliorated completely the Alloxan effect on serum levels of glucose, cholesterol, triglyceride, creatinine and urea in Alloxan-induced diabetic rabbits. GHA and Glimepiride (a reference drug) partially blocked such effect of the Alloxan in treated rabbits. Further GH, GHA and GHE did not cause any change in food/water intakes and on liver, spleen, kidney, lung and heart in treated rabbits. Phytochemical study of GH and its extracts revealed the presence of flavonoids and phenolic compounds. Histopathological examination showed the protective effect of GH, GHA and GHE against Alloxan-induced destruction of β-cells of pancreas in diabetic rabbits. Data indicated that GH and its aqueous and ethanol extracts have promising anti-diabetic and hypolipidemic effects. GH and GHE could be effective tool against the development, progression and complication of *Diabetes mellitus*.

Keywords: Anti-diabetic, hypolipidemic, hypoglycemic, *Gossypium herbaceum.*

INTRODUCTION

Seed of *Gossypium herbaceum* L (GH) is a well-known traditional/herbal medicine. GH is commonly used as food, as well as medicine in a number of formulations (Batur *et al*., 2008). It is a good source of vitamins and is an excellent analgesic. Gossypin, isolated from GH has been found analgesic and anti-inflammatory (Gupta and De, 2012). Vitamin-E is obtained from leaf of GH. Seed oil is often used as a substitute for sesame oil in cooking. Cottonseed (GH) oil is also used as cooking oil, as well as salad oil and shortening. In addition, it can produce thicker, longer-lasting soapsuds and this oil is found in many soap products (Zaman *et al.*, 2011). Extract of bark and root of GH, has been reported to strengthen the uterine muscular contractions. It has been used for the ease of childbirth, to induce miscarriages and to stimulate irregular menstrual cycles successfully. Further it has been found useful to treat the menopause symptoms, like hot flashes. Root bark decoction is effectively employed for the treatments of urinary disorders. The leaf decoction is useful for relief of headaches and fever. Further leaf and seed oil are helpful for treating snakebites and skin disorders, such as poison ivy and warts (Srivastava *et al.*, 2012). Velmurugan and Bhargava, (2014) have been reported the anti-diabetic activity of leaf of *Gossypium herbaceum*in rat models. GH chiefly possesses antifertility, galactagogue, antispermatogenic, anti-diabetic, antiviral and antibacterial activity (Rahaman *et al.,* 2012).

In addition to hyperglycaemia and muscular weakness, *Diabetes mellitus* (DM) is caused the onset of polyphagia, polydipsia, polyuria and gradual weight-loss. Cellular membranes of different tissue of body organs such as retina of eye, glomeruli in kidney, erythrocyte and nerve are altered during the development and progression of both Type-1 and Type-2 DM leading to the alterations in the cell structures, their organizations and protein functions. Hence pathogenic course of DM culminates in diabetic complications including cardiopathy, retinopathy, nephropathy and peripheral neuropathy (Zaman *et al.*, 2011).

Plants have been found a major source of drugs. A number of currently used drugs have been obtained either directly or indirectly from herbal source. Herbal treatment of DM has been indicated many plants/herbs with the promising significance. Several such plants have been reported to be hypoglycaemic both individually as well as in combinations (Wadood *et al.*, 2003). On the other hand, a few safe modern drugs are available for the treatment of DM. Therefore, WHO has been recommended for the evaluation of safe and effective medicinal plants for the treatment of disorders like diabetes (Kim *et al.*, 2007). Consequently, anti-diabetic herbal products with lesser

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side effects are with an increasing demand (Hasani-Ranjbar *et al*., 2008). Therefore, present study was design to examine anti-diabetic and hypolipidemic effect of *Gossypium herbaceum* L. seed and its aqueous and ethanol extracts in Alloxan-induced diabetic rabbit models.

MATERIALS AND MATHODS

Plant material and preparation of extracts

Gossypium herbaceum L seed (GH) was purchased from leading herbal dealer in Bahawalpur-Pakistan and seed was identified and authenticated by the expert taxonomist. A specimen sample was preserved (voucher # GH 063-12-02-2010) at the Pharmacology Section, Department of Pharmacy, the Islamia University of Bahawalpur-PAKISTAN, for future reference. Seeds were dried under the shade at room temperate before ground to fine powder (75μ). Aqueous and ethanol (95%) extracts (GHA, yield 119.3g; 11.93% and GHE, yield 89.6g; 8.96% respectively) were prepared separately by maceration (1.0kg/1.5L) at room temperature. Collected supernatants were concentrated under reduced pressure in a rotary evaporator (Laborota, Heidolph) at 37^{o}C separately. Dried extracts were stored in a refrigerator before commencement of studies (Zaman and Rehman, 2010).

Chemicals

All chemicals used; Alloxan monohydrate and Sodium carbonate, Sodium citrate were obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA). Test kits including glucose estimation kit (Diagnostics Elitech kit) were obtained from ELISA, Boerhringer Mannheim, Germany. The reference anti-diabetic drug, Glimepiride was taken from Ferozsons Laboratories Limited, Rawalpindi, Pakistan.

Experimental animals

Adult healthy male rabbits (*Oryetolagus cuniculus*) of local bread, weighing about 1.20-1.50kg were acclimatized under the standard conditions of temperature (23±12^{o}C), humidity (55±15%) and light (7.00-19.00). Animals were fed according to a strict schedule (6.00, 14.00 and 20.00h) with a free access to a balanced rabbit's diet consisting of green leaves, fodder, pulses (*Medicago sativa*) and water *ad libitum*. Overnight fasted animals were randomly divided into different groups (6-8 animals per group) and they were used in accordance to the guidelines of NIH for the care and use of laboratory animals in the present study (Zaman, 2011).

Diabetes mellitus induction

A single intravenous (*i.v.*) injection of Alloxan monohydrate (100mg/kg, b.w., dissolved in 0.1 M Sodium citrate buffer pH 4.5) was given to induce *Diabetes mellitus* (DM) in rabbits. Control group received similar volume of the vehicle (citrate buffer, 1.0ml/kg). Alloxan-treated rabbits received 5% of glucose instead of water for next 24h, in order to reduce the possibility of death due to hypoglycemic shock (Barbosa *et al*., 2008).

Administration of drugs

On experimental day-01 different groups of overnight-fasted normal rabbits were treated as follows:

Group 1: Lactose 300mg/kg, b.w. *p.o*. Group 2: GH 200mg/kg, b.w. *p.o*. Group 3: GH 250mg/kg, b.w. *p.o*. Group 4: GH 300mg/kg, b.w. *p.o*. Group 5: GHA equivalent to 300mg/kg powdered GH, b.w. *p.o*. Group 6: GHE equivalent to 300mg/kg powdered GH, b.w. *p.o*. Group 7: Alloxan 100mg/kg, b.w. *i.v*. Group 8: GH 200mg/kg, b.w. *p.o*. + Alloxan 100mg/kg, b.w. *i.v*. Group 9: GH 250mg/kg, b.w. *p.o*. + Alloxan 100mg/kg, b.w. *i.v*. Group 10: GH 300mg/kg, b.w. *p.o*. + Alloxan 100mg/kg, b.w. *i.v*. Group 11: GHA equivalent to 300mg/kg powdered GH, b.w. *p.o*. + Alloxan 100mg/kg, b.w. *i.v*. Group 12: GHE equivalent to 300mg/kg powdered GH, b.w. *p.o*. + Alloxan 100mg/kg, b.w. *i.v*. Group 13: Glimepiride 0.1mg/kg, b.w. *p.o*. + Alloxan 100mg/kg, b.w. *i.v*.

All the treated, toxic-control and reference-control rabbits were injected with a single dose of Alloxan monohydrate (C4H2N2O4.H2O, 10%) 100mg/kg, b.w. intravenously (*i.v.*) while an equal volume of vehicle (*i.v.*) was given to all other rabbits one hour later of respective oral treatments. Oral treatments were continued 1-21 days (12 hourly). In order to evaluate behavioral and toxic effects of test substances; GH, GHA and GHE were given to the different groups of overnight-fasted rabbits in very high doses *i.e.* 0.3, 0.5 and 1.0g/kg, b.w. *p.o.* separately (Zaman and Rehman, 2010).

Laboratory analyses

Blood samples from all the rabbits under test were collected before the start of treatments (day-1) and on 8, 15 and 22 experimental days. Specimens were centrifuged at 6000rpm for 15min. Serum was used for the glucose, total cholesterol, triglyceride, creatinine and urea estimation on Micro lab (7170S, HITACHI) by using the commercial kits (Kaleem *et al*., 2006).

Water intake and food consumption

Post treatment, intake of water and consumption of food was measured on 1, 8, 15 and 22 experimental days.

Organ weight (Liver, Spleen, Lung, Kidney and Heart)

Body weights of animals were measured pre (day-1) and post treatments before their scarification (day-22). Following collection of blood samples, the animals were dissected and their liver, spleen, lung, kidney and heart were extracted out to weigh and to evaluate macroscopically (Zaman *et al*., 2011).

Table 1: Effects of *Gossypium herbaceum* L powdered seeds and its extracts on serum glucose level in normal rabbits

Group No.	Treatment(s)	Serum glucose concentration (mg/dl) on day			
		1	8	15	22
01	Lactose, 300 mg/kg (Normal control)	134.3±3.12	134.0±3.01	133.5±3.08	133.9±3.01
02	*G. herbaceum* powdered seeds (200 mg/kg)	137.0±1.28	133.9±1.16	132.1±1.30*	131.2±1.33*
03	G. herbaceum powdered seeds (250 mg/kg)	137.0±1.28	135.9±1.16	133.1±1.30*	130.2±1.33*
04	*G. herbaceum* powdered seeds (300 mg/kg)	137.0±1.28	133.9±1.16	130.1±1.30*	127.2±1.33*
05	GHA (eq. to 300 mg/kg of GH)	137.0±1.28	136.9±1.41	136.6±1.40	136.7±1.37
06	GHE (eq. to 300 mg/kg of GH)	137.0±1.28	135.4±1.23	129.4±1.23*	126.5±1.42*

Table 2: Effects of *Gossypium herbaceum* L powdered seeds and its extracts on serum glucose level in diabetic rabbits

Group No.	Treatment(s)	Serum glucose concentration (mg/dl) on day			
		1	8	15	22
01	Lactose, 300 mg/kg (Normal control)	134.3 ±3.12	134.1±3.01	133.5±3.08	133.9±3.01
07	Alloxan (100 mg/kg) (Diabetic Control)	133.7±4.30	198.1±4.12**	225.2±11.2**	227.0±11.49**
08	Alloxan (100 mg/kg) + GH (200 mg/kg)	135.3±3.22	161.6±5.14*	187.2±11.15*	172.6±7.63*
09	Alloxan (100 mg/kg) + GH (250 mg/kg)	135.6±4.38	145.6±4.41	167.7±8.55*	157.9±4.64*
10	Alloxan (100 mg/kg) + GH (300 mg/kg)	132.1±2.42	144.1±4.99	146.2±5.88	134.4±1.13
11	Alloxan (100 mg/kg) + AGH (eq. to 300 mg/kg)	133.3±1.34	154.6±4.49*	179.3±10.39*	169.6±7.81*
12	Alloxan (100 mg/kg) + EGH (eq. to 300 mg/kg)	135.4±3.43	140.6±1.17	141.8±2.15	134.8±1.33
13	Alloxan (100 mg/kg) + Glimepiride (0.1 mg/kg)	136.7±2.64	159.6±4.63*	182.8±9.79*	173.4±7.63*

Table 3: Effects of *Gossypium herbaceum* L powdered seeds and its extracts on serum cholesterol level in diabetic rabbits

Group No.	Treatment(s)	Serum cholesterol concentration (mg/dl) on day			
		1	8	15	22
01	Lactose, 300 mg/kg (Normal control)	167.3±2.46	167.3±2.21	167.5±2.21	167.4±2.11
07	Alloxan (100 mg/kg) (Diabetic Control)	169.6±2.12	186.1±5.11*	216.7±9.21**	241.1±10.23**
08	Alloxan (100 mg/kg) + GH (200mg/kg)	165.5±5.19	176.2±3.74	191.8±7.41*	187.6±3.24*
09	Alloxan (100 mg/kg) + GH (250mg/kg)	167.4±3.13	171.4±2.11	183.2±7.45*	179.2±4.14*
10	Alloxan (100 mg/kg) + GH (300mg/kg)	167.2±4.32	171.8±4.59	174.3±5.24	170.3±1.11
11	Alloxan (100 mg/kg) + AGH (eq. to 300 mg/kg)	164.5±2.28	167.4±1.16	198.9±6.25*	199.3±3.99*
12	Alloxan (100 mg/kg) + EGH (eq. to 300 mg/kg)	166.9±3.10	169.2±1.50	172.7±3.11	168.7±1.24
13	Alloxan (100 mg/kg) + Glimepiride (0.1 mg/kg)	169.6±2.21	173.7±1.31*	193.1±5.48**	198.4±3.46*

Table 4: Effects of *Gossypium herbaceum* L powdered seeds and its extracts on serum triglyceride level in diabetic rabbits

Group No.	Treatment(s)	Serum cholesterol concentration (mg/dl) on day			
		1	8	15	22
01	Lactose, 300 mg/kg (Normal control)	131.6±1.261	131.7±1.011	131.5±1.432	131.6±1.256
07	Alloxan (100 mg/kg) (Diabetic Control)	128.3±2.168	146.8±1.32**	187.9±4.57**	205.3±3.864**
08	Alloxan (100 mg/kg) + GH (200mg/kg)	127.5±3.338	141.3±1.202*	168.4±8.783*	169.6±4.612*
09	Alloxan (100 mg/kg) + GH (250mg/kg)	130.1±4.123	140.2±2.874	155.7±3.99**	157.3±2.111*
10	Alloxan (100 mg/kg) + GH (300mg/kg)	129.8±2.411	135.0±2.198	137.6±3.795*	131.3±1.091
11	Alloxan (100 mg/kg) + AGH (eq. to 300 mg/kg)	129.4±3.214	145.9±3.224*	165.8±7.953*	162.1±4.211*
12	Alloxan (100 mg/kg) + EGH (eq. to 300 mg/kg)	127.2±3.467	131.1±1.511	135.8±4.522	126.1±1.817
13	Alloxan (100 mg/kg) + Glimepiride (0.1 mg/kg)	130.6±3.324	146.3±3.165*	164.9±5.78**	161.9±3.416*

GH: *Gossypium herbaceum* L powdered seed, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seed, EGH: Ethanol extract of *Gossypium herbaceum* L powdered seed, eq. to: equivalent to, Mean ±S.E.M = mean ±standard error of mean of six rabbits, Test drugs: significant from respective normal values obtained onday-1 (pre-treatment), *P<0.05, **P<0.001, All other values are non-significant (P>0.05) from respective normal values

Table 5: Effects of *Gossypium herbaceum* powdered L seeds and its extracts on serum creatinine level in diabetic rabbits

Group No.	Treatment(s)	Serum creatinine level (mg/dl) on day			
		1	8	15	22
01	Lactose, 300 mg/kg (Normal control)	0.681±0.012	0.682±0.021	0.671±0.023	0.672±0.010
07	Alloxan (100 mg/kg) (Diabetic Control)	0.744±0.154	1.253±0.022*	1.551±0.05**	2.796±0.346**
08	Alloxan (100 mg/kg) + GH (200mg/kg)	0.674±0.124	0.997±0.073	1.681±0.132*	1.380±0.111*
09	Alloxan (100 mg/kg) + GH (250mg/kg)	0.652±0.044	0.884±0.117	1.294±0.24*	1.252±0.101*
10	Alloxan (100 mg/kg) + GH (300mg/kg)	0.664±0.161	0.821±0.011	1.116±0.182	0.887±0.055
11	Alloxan (100 mg/kg) + AGH (eq. to 300 mg/kg)	0.637±0.041	0.971±0.153	1.659±0.289*	1.100±0.109*
12	Alloxan (100 mg/kg) + EGH (eq. to 300 mg/kg)	0.682±0.112	0.823±0.043	1.181±0.154	0.860±0.128
13	Alloxan (100 mg/kg) + Glimepiride (0.1 mg/kg)	0.634±0.138	0.992±0.032	1.570±0.217*	1.221±0.021*

Table 6: Effects of *Gossypium herbaceum* L powdered seeds and its extracts on serum urea level in diabetic rabbits

Group No.	Treatment(s)	Serum urea level (mg/dl) on day			
		1	8	15	22
01	Lactose, 300 mg/kg (Normal control)	27.51±1.106	27.44±1.212	27.41±1.741	27.43±1.108
07	Alloxan (100 mg/kg) (Diabetic Control)	27.12±1.202	34.54±1.310*	41.78±2.14**	54.07±1.311**
08	Alloxan (100 mg/kg) + GH (200mg/kg)	25.40±1.354	29.87±1.529	36.31±1.01**	31.85±2.353*
09	Alloxan (100 mg/kg) + GH (250mg/kg)	26.88±1.345	30.91±1.356	30.81±3.841	30.04±1.873
10	Alloxan (100 mg/kg) + GH (300mg/kg)	26.88±1.345	30.91±1.356	30.81±3.841	30.04±1.873
11	Alloxan (100 mg/kg) + AGH (eq. to 300 mg/kg)	23.61±1.311	28.16±1.755	44.94±2.21**	39.63±1.511*
12	Alloxan (100 mg/kg) + EGH (eq. to 300 mg/kg)	28.06±2.474	30.23±0.223	30.12±2.112	29.01±0.410
13	Alloxan (100 mg/kg) + Glimepiride (0.1 mg/kg)	24.53±1.323	29.18±1.756	44.91±2.24**	41.06±2.152**

GH: *Gossypium herbaceum* L powdered seed, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seed, EGH: Ethanol extract of *Gossypium herbaceum* L powdered seed, eq. to: equivalent to, Mean ±S.E.M = mean ±standard error of mean of six rabbits, Test drugs: significant from respective normal values obtained onday-1 (pre-treatment), *P<0.05, **P<0.001, All other values are non-significant (P>0.05) from respective normal values

Table 7: Behavioural effects of *Gossypium herbaceum* L powdered seeds, it's aqueous and ethanol extracts in rabbits (day 1-22)

S. No.	Treatment(s)	Body weight	Water intake	Food intake	Diarrhoea	Movements	Convulsion	Sleep
01	GH (300mg/kg)	N	N	N	No	N	No	N
02	GH (500mg/kg)	N	N	N	No	N	No	N
03	GH (1.0g/kg)	N	N	N	No	N	No	N
04	AGH (eq. to 300mg/kg of GH)	N	N	N	No	N	No	N
05	AGH (eq. to 500mg/kg of GH)	N	N	N	No	N	No	N
06	AGH (eq. to 1.0g/kg of GH)	N	N	N	No	N	No	N
07	EGH (eq. to 300mg/kg of GH)	N	N	N	No	N	No	N
08	EGH (eq. to 500mg/kg of GH)	N	N	N	No	N	No	N
09	EGH (eq. to 1.0g/kg of GH)	N	N	N	No	N	No	N

Table 8*:* Toxic effects of *Gossypium herbaceum* L powdered seeds, it's aqueous and ethanol extracts in rabbits (day 1-22)

S. No.	Treatment(s)	Lethality after day			Macroscopic changes after 22 days in			
		8	15	22	Liver Spleen	Kidney	Lungs	Heart
01	GH (300 mg/kg)	No	No	No	N	N	N	N
02	GH (500 mg/kg)	No	No	No	N	N	N	N
03	GH (1.0 g/kg)	No	No	No	N	N	N	N
04	AGH (eq. to 300 mg/kg of GH)	No	No	No	N	N	N	N
05	AGH (eq. to 300 mg/kg of GH)	No	No	No	N	N	N	N
06	AGH (eq. to 1.0 g/kg of GH)	No	No	No	N	N	N	N
07	EGH (eq. to 300 mg/kg of GH)	No	No	No	N	N	N	N
08	EGH (eq. to 500 mg/kg of GH)	No	No	No	N	N	N	N
09	EGH (eq. to 1.0 g/kg of GH)	No	No	No	N	N	N	N

GH: *Gossypium herbaceum* L powdered seeds, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seeds, GH: Ethanol

Table 9: Pancreatic histopathological changes induced by *Gossypium herbaceum* L powdered seeds, its aqueous and ethanol extracts in Alloxan-treated rabbits.

Group No	Treatment	Pancreatic β-cells			
		Number	Shrinkage	Necrosis	Repair
1	Normal	N	--	--	N
7	Alloxan (100 mg/kg)	L	+++	+++	--
8	Alloxan (100 mg/kg) + GH (200 mg/kg)	L	++	+	--
9	Alloxan (100 mg/kg) + GH (250 mg/kg)	L	+	--	--
10	Alloxan (100 mg/kg) + GH (300 mg/kg)	N	--	--	N
11	Alloxan (100 mg/kg) + AGH (eq. to 300 mg/kg)	L	++	+	--
12	Alloxan (100 mg/kg) + EGH (eq. to 300 mg/kg)	N	--	--	N
13	Alloxan (100 mg/kg) + Glimepiride (0.1 mg/kg)	L	++	+	--

N: normal, L: less in number, +: mild, ++: moderate, +++: sever, GH: *Gossypium herbaceum* L powdered seed, AGH: aqueous extract of GH, EGH: ethanol extract of GH

Histopathological examination

All the animals were sacrificed on experimental day-22 and their pancreas were removed. Isolated tissues were fixed in 10% formalin and processed for paraffin embedding. Tissues were sliced 6mm in thickness. Paraffin wax-embedded slices of pancreas were sectioned at 5μm. Sections were stained with haematoxylin, eosin and were mounted in Canada balsam. Histopathological assessment was done according to the standard method (Garba *et al.*, 2009).

Table 10: Phytochemical Analysis of *Gossypium herbaceum* L powdered seeds and its extracts.

Tested for	GH	AGH	EGH
Alkaloids	+	+	+
Tannins	+	--	+
Anthraquinones	--	--	--
Flavonoids	++	+	++
Phenolic compounds	++	+	++
Glycosides	--	--	--
Cardiac glycosides	--	--	--
Steroids	+	+	+
Saponins	+	+	+
Coumarins	+	+	+

--: absent; +: present; ++: strongly present.

STATISTICAL ANALYSIS

Mean values of six experiments ± SEM (Standard Error of Means) were analyzed for statistical significance by one-way ANOVA. Findings were found significant at $p<0.05$ (Zaman *et al.*, 2011).

RESULTS

Effects of Gossypium herbaceum* L *seed and its extracts in normal rabbits

Hypoglycemic effect

GH, GHA and GHE caused significant ($P<0.05$) hypoglycemic effect in the normal rabbits. Increasing hypoglycemic effects were observed with the increase in dose (200-300mg/kg, b.w.) of GH (table 1).

Effect on cholesterol, triglyceride, urea and creatinine serum levels

A decrease in cholesterol, triglyceride and urea serum levels while no effect on creatinine level was observed in normal rabbits treated with GHA, GH and GHE (fig. 1).

Effect of Gossypium herbaceum* L *seed powder and its extracts in Alloxan-treated diabetic rabbits

Effect on glucose serum level

Alloxan-treated diabetic rabbits were shown high significant ($P<0.001$) increases in glucose serum level (table 2). This hyperglycemic effect was antagonized high significantly ($P<0.001$) by GH and GHE in a dose dependent manner (day 1-22). The effect was attenuated significantly ($P<0.05$) by GHA and reference drug Glimepiride. Data showed that GHE lowered blood glucose levels in all diabetic rabbits, and its anti-hyperglycemic activity was better than with all other test agents (table 2).

Effect on cholesterol, triglyceride, creatinine and urea serum levels

Alloxan-treatment induced a high significant ($P<0.001$) increase in cholesterol, triglyceride, creatinine and urea serum levels in the diabetic rabbits (table 3-6). GHE enumerated completely the effect of Alloxan on cholesterol, triglyceride, creatinine and urea serum levels in a dose dependent manner (day 1-22) in the treated rabbits. However, GHA and Glimepiride decreased significantly ($P<0.05$) such effects of Alloxan in treated and reference-control rabbits respectively (table 3-6).

Effect of GH, GHA and GHE on water intake and food consumption in rabbits

Effect of GH and its aqueous and ethanol extracts on water and food consumption is shown in the table 7. Data showed that GH, GHA and GHE caused no significant effect on intake of water and food in treated-rabbits (table 7).

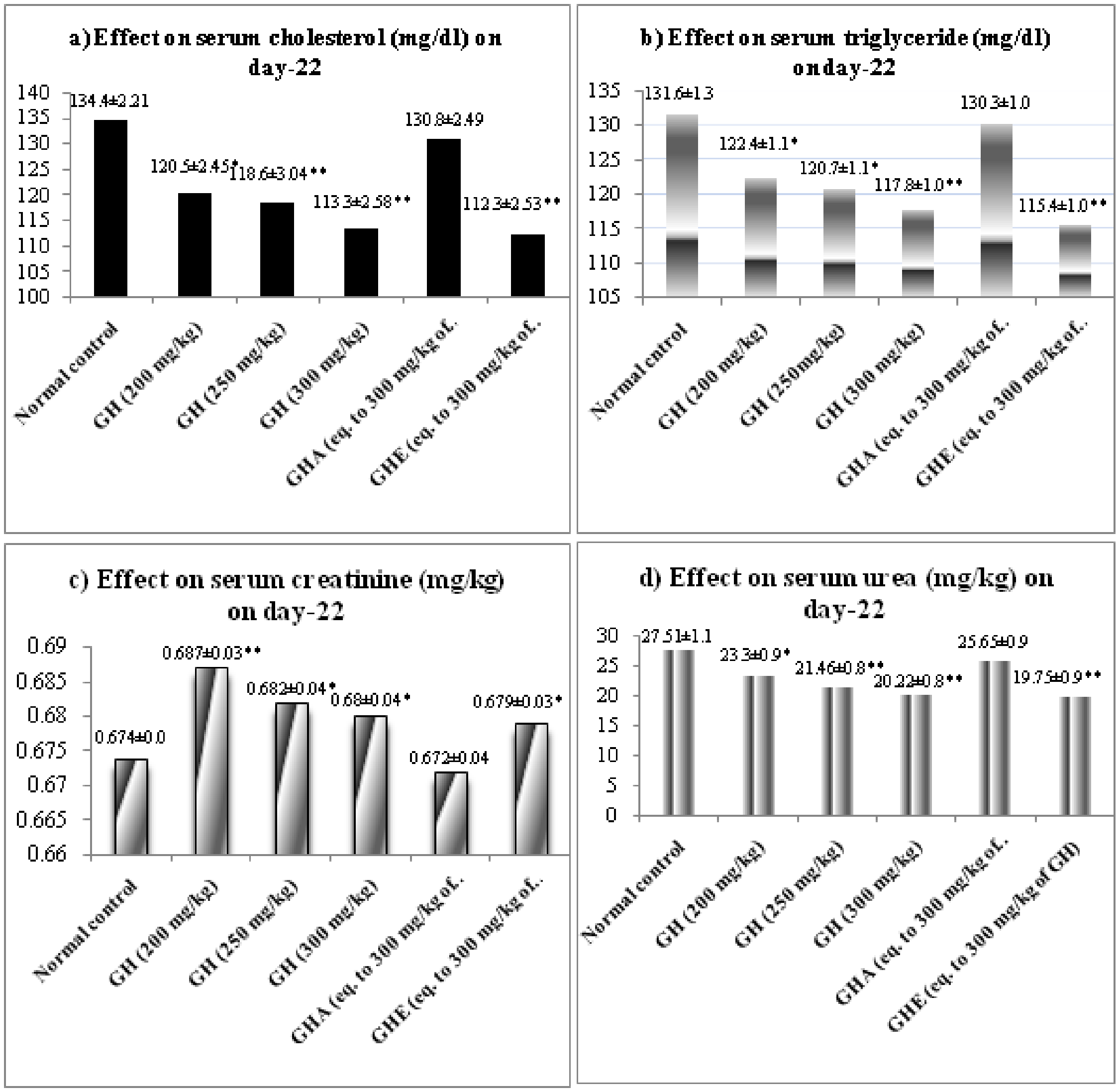

Fig. 1: Effect of GH, GHA and GHE on serum cholesterol, triglyceride, urea and creatinine levels in normal rabbits

GH: *Gossypium herbaceum* L powdered seeds, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seeds, EGH: Ethanol extract of *Gossypium herbaceum* L powdered seeds, eq. to: equivalent to, Mean ±S.E.M = mean ±standard error of mean of six rabbits, Test drugs: significant from normal control, *P<0.05, **P<0.001, All other values are non-significant (P>0.05) from respective normal values, GH: *Gossypium herbaceum* L powdered seeds, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seeds, EGH: Ethanol extract of *Gossypium herbaceum* L powdered seeds, eq. to: equivalent to

Effect of GH, GHA and GHE on Liver, Spleen, Lung, Kidney and Heart in rabbits

Table 8 showed the effects of GH, GHA and GHE on liver, spleen, lung, kidney and heart of treated rabbits. Liver, spleen, lung, kidney and heart were found normal following administration of high doses (300mg/kg-1.0g/kg, b.w.) of GH, GHA or GHE for 21 days in rabbits (table 8).

Histopathological effect of GH, GHA and GHE on pancreatic β-cells in diabetic rabbits

Table 9 summarizes the amelioration of Alloxan-induced histopathological changes by GH, GHA and GHE in diabetic rabbits. GH and GHE attenuated almost completely while GHA and reference drug, Glimepiride antagonized partially such histopathological changes in the treated rabbits (fig. 2-7)

Phytochemical analysis of Gossypium herbaceum* L *seed and its extracts

Table 10 shows phytochemical analysis of GH, GHA and GHE. GH: *Gossypium herbaceum* L powdered seeds, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seeds, EGH: Ethanol extract of *Gossypium herbaceum* L powdered seeds, eq. to: equivalent to, Mean ±S.E.M = mean±standard error of mean of six rabbits, Test drugs: Significant from normal control, *P<0.05, **P<0.001, All other values are non-significant (P>0.05) from respective normal values, GH: *Gossypium herbaceum* L powdered seeds, AGH: Aqueous extract of *Gossypium herbaceum* L powdered seeds, EGH: Ethanol extract of *Gossypium herbaceum* L powdered seeds.

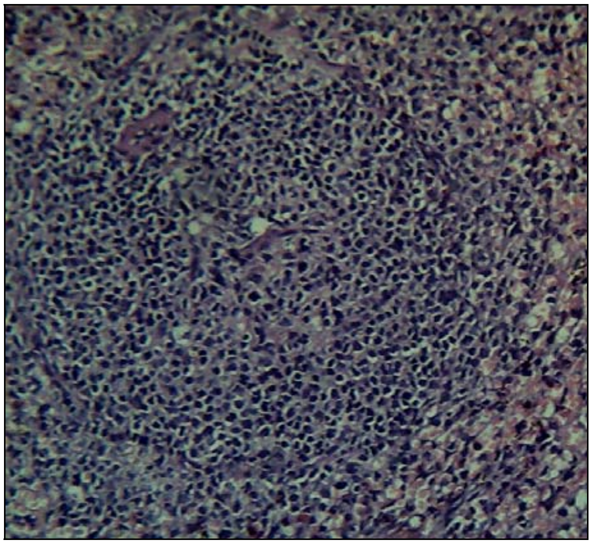

Fig. 2: β-cells of pancreatic Islet of Langerhans of Normal control rabbits

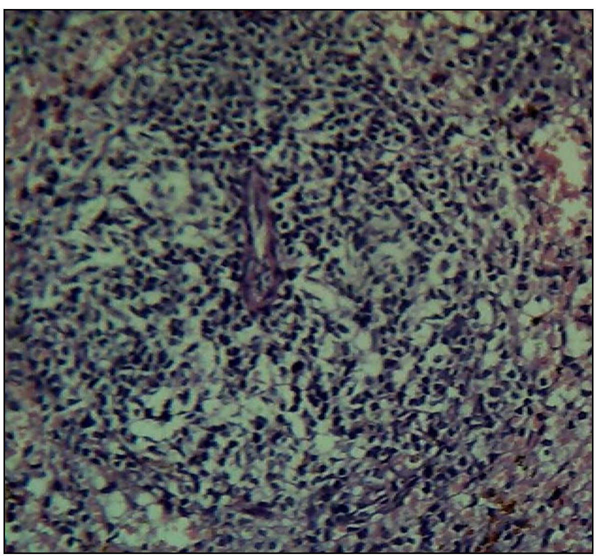

Fig. 3: Alloxan (100mg/kg) induced damage in pancreatic β-cells in diabetic control rabbits

DISCUSSION

In addition to hyperglycaemia and muscular weakness, *Diabetes mellitus* (DM) is caused the onset of polyphagia, polydipsia, polyuria and gradual body weight-loss. Present study evaluated the effect of aqueous and ethanol extracts of *Gossypium herbaceum* L seed (GH) on different parameters such as; Glucose, triglycerides, cholesterol, creatinine and urea serum levels in Alloxan induced diabetic rabbits. Water/food intake and toxic effect were also observed in the rabbits.

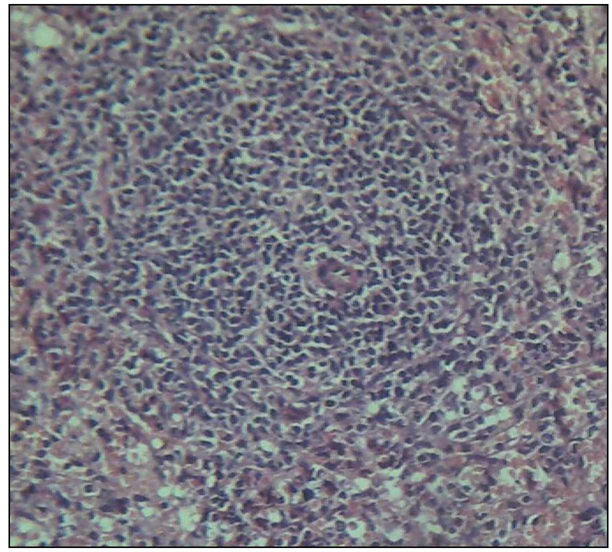

Fig. 4: Complete inhibition on pancreatic β-cells caused by GH in Alloxan-treated rabbits

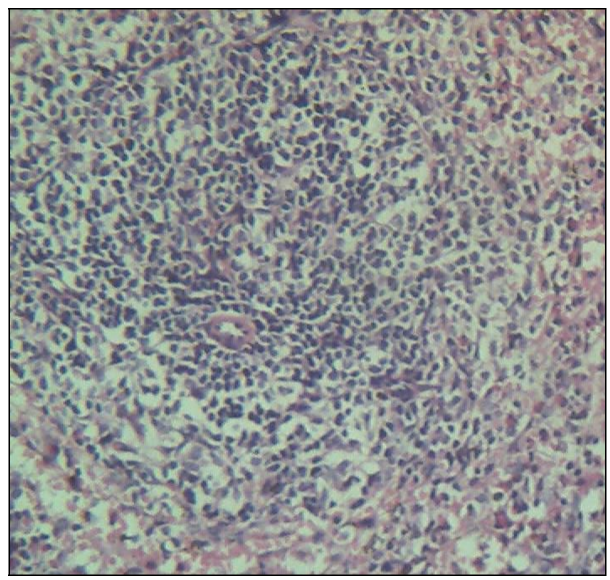

Fig. 5: Partial protection by GHA (eq. to 300mg/kg GH) in Alloxan-treated rabbits

Alloxan is a highly toxic substance, which is frequently used for the induction of *Diabetes mellitus* (DM) in various animal models (Sharma *et al.*, 2010a). It is caused destruction of the pancreatic β-cells and causes diabetes by inducing cell necrosis. Reactive oxygen species generation due to cytotoxic activity of Alloxan has been reported to be involved in the onset of the disease (Khushk *et al.*, 2010). A redox cycle is established due to the formation of superoxide radicals by this diabetogenic agent and its metabolic product, dialuric acid. In addition, a simultaneous massive rise of Ca^{+2} concentrations in cytosol causes rapid destruction of pancreatic β-cells and induction of hyperglycemia (Luo *et al.*, 2004; Jyoti *et al.*, 2013).

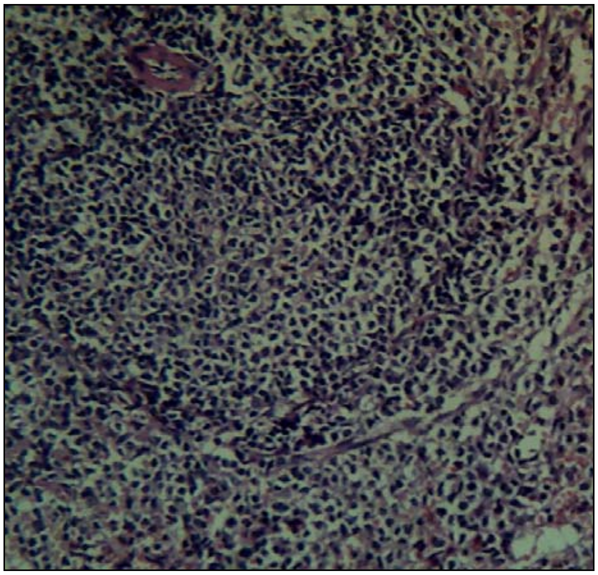

Fig. 6: Preventive effects of GHE (eq. to 300mg/kg GH) in Alloxan-treated rabbits

Present study showed that intravenous administration of Alloxan (100mg/kg, b.w.) markedly induced DM in diabetic-control rabbits as reflected by hyperglycemia, polyphagia, polydipsia, polyuria and body weight-loss compared with the normal control rabbits. Table 2 shows a high significant (P<0.001) hyperglycemic activity in Alloxan treated rabbits on day-22 in comparison to day-1 (Mahesar *et al.*, 2010; Jyoti *et al.*, 2013). A dose dependent reduction in glucose serum level was observed with GH 200, 250 and 300mg/kg, b.w., while 300mg/kg dose caused a complete prevention against Alloxan effect and almost normal glycemia was observed in comparison to their respective day-1 values (table 2). Ethanol extract of *Gossypium herbaceum* (GHE) equivalent to 300mg/kg, b.w. showed highly significant (P<0.001) decline in glucose serum level as compared to the diabetic-control rabbits (day 1-22). The serum glucose level in GHE treated rabbits was non-significant (P>0.05) in comparison to the normal-control rabbits (table 2). This effect of GHE was found to be in agreement to the observations of Mahesar *et al*., 2010. GH (200 and 250mg/kg) showed the significant (P<0.05) glucose lowering effect (table 2). A significant (P<0.05) fall in glucose serum concentration was found in diabetic-rabbits treated with aqueous extract of GH (GHA, equivalent to 300mg/kg) and reference drug, Glimepiride, as compared to diabetic-control (day 1-22, table 2). The observed effect of Glimepiride is in-agreement with the findings of Mir *et al*., 2008. Present study reveals that diabetic-rabbits treated with GH 300mg/kg and GHE equivalent to 300mg/kg of GH, lowered the glucose serum level high significantly (P<0.001) as compared to the diabetic-control and glucose serum level remained normal from day 1-22 (table 2).

Hypercholesterolemia and hypertriglyceridemia were also observed in Alloxan diabetic rabbits (day 1-22, table 3 and 4). These observations are agreed with the findings of Ahmad *et al*., 2009. A high concentration of serum lipids in DM has been identified due to deficiency of insulin, which yields potent inhibitory effect on lipolysis in adiposities (Gohil *et al.*, 2010). Alloxan caused a highly significant (P<0.001) elevation in the serum level of cholesterol in diabetic-control as compared to the normal-control rabbits within 21 days. A similar effect was reported by Ahmad *et al*., (2009). GH 200 and 250mg/kg caused partial antagonism against Alloxan induced hypercholesterolemia (day 1-22, table 3). The elevation in cholesterol serum level post-treatment of diabetic rabbits with GH 300mg/kg and GHE was completely retarded and the effects were non-significant (P>0.05) in comparison to diabetic-control (Kumar and Loganathan, 2010). Glimepiride caused a significant (P<0.05) fall in cholesterol serum level as compared to diabetic-control (day 1-22, table 3) (Yassin and Mwafy, 2007; Kumar and Loganathan, 2010). The result suggests that the GH 300mg/kg and GHE (equivalent to 300mg/kgof GH) have greater anti-hypercholesterolemia potential in diabetic rabbits as compared to the Glimepiride, AGH and GH (200 and 250mg/kg).

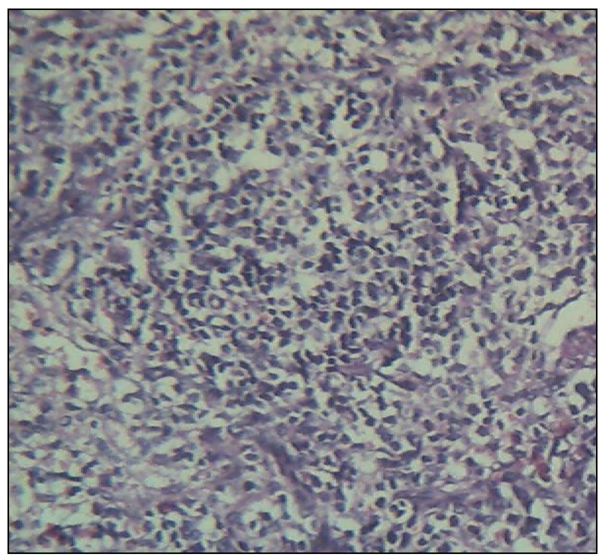

Fig. 7: Partial attenuation caused by Glimepiride in Alloxan-treated rabbits

Hypertriglyceridemia has been reported to occur in Alloxan diabetic rabbits (Ahmad *et al*., 2009). The report is compatible with the present study where the highly significant (P<0.001) elevation in the triglyceride serum level in Alloxan treated rabbits was observed (table 4). GH caused anti-hypertriglyceridemic effect in a dose-dependent (200, 250 and 300mg/kg) manner in treated rabbits (day 1-22, table 4). GH in 300mg/kg dose caused a complete blockage of the hypertriglyceridemic effect in Alloxan treated rabbits in comparison to the diabetic-control rabbits (table 4). GHE attenuated the increase in triglyceride serum level high significantly (P<0.001) in treated rabbits as compared to the diabetic-control rabbits (day 1-22). This finding is non-significant (P>0.05) in comparison to the normal-control group (Luo *et al.*, 2004). A fall in the triglyceride serum level with GHA and Glimepiride was found significant (P<0.05) (Yassin and Mwafy, 2007). It is obvious from the result of present study that GHE caused greater reduction in triglyceride level than GHA and Glimepiride (table 4). It is well known that the level of glycemia is the major determinant of the serum level of triglycerides (Kumar and Loganathan, 2010). Several investigators demonstrated that the normalization of blood glucose resulted in significant reduction in cholesterol and triglyceride serum levels (Kumar and Loganathan, 2010). Our finding is in-agreement with such observations. The treatment with GHE and GH 300mg/kg in diabetic rabbits showed that serum concentrations of glucose, cholesterol and triglyceride were high significantly (P<0.001) decreased (day-8, 15, 22). Therefore, data indicates sufficiently that *Gossypium herbaceum* L might prove an important tool in treating diabetic/hyperlipidemic disorders (Darvhekar *et al.*, 2013; Velmurugan and Bhargava, 2014).

DM is frequently linked with severe complications following the onset of disease. Most commonly it involves urinary tract leading to renal dysfunction which can be accessed by creatinine and urea serum levels (Daisy *et al.*, 2009). Data showed a significant rise in creatinine and urea serum levels in the diabetic animals which indicated impairment of renal function caused by Alloxan treatment (Daisy *et al.*, 2009). Nwozo *et al.*, (2009) indicated a significant (P<0.001) increase in creatinine synthesis by DM. This observation is consistent with the findings of present study where Alloxan induced diabetes and a highly significant (P<0.001) elevation in the creatinine level was found (table 5) (Garg *et al.*, 2009). GH caused a fall in serum creatinine concentration in a dose dependent order (200-300mg/kg, b.w.) in diabetic rabbits (day 1-22, table 5). Maximum dose of GH, 300mg/kg, established a complete reversal of rise in creatinine serum level as compared to diabetic-control group (table 5). GHE showed a complete restoration and a highly significant (P<0.001) fall in serum creatinine concentration in comparison to the diabetic-control rabbits. This finding is non-significant (P>0.05) as compared to the normal-control rabbits (Daisy *et al.*, 2009; Nwozo *et al.*, 2009). GHA and Glimepiride also exhibited significant (P<0.05) fall in creatinine serum level as compared to diabetic-control (table 5). The effect induced by GH, 300mg/kg and GHE (equivalent to 300mg/kgof GH) was greater than AGH (equivalent to 300mg/kgof GH) and Glimepiride (table 5).

A high rate of protein catabolism as well as deamination of amino acid for DM induced gluconeogenesis is probably an acceptable postulate to explain the increased serum level of urea (Yassin and Mwafy, 2007). Data showed a high significant (P<0.001) rise in urea serum level (day-22) induced by Alloxan in diabetic rabbits (table 6) (Ananthi *et al.*, 2003). GH 200 and 250mg/kg, b.w. treatments in diabetic rabbits caused significant (P<0.05) attenuation in urea level while GH 300mg/kg and GHE offered a high significant (P<0.001) reduction in urea level as compared to diabetic-control animals (table 6). This fall in urea serum level at day-22 caused by GHE was noted comparable with the urea concentration on day-1. The effect of GHE was similar to that of ethanol extract of *Caralluma sinaica* evaluated by Habibuddin *et al.*, 2008. The effect of GHA and Glimepiride were comparable with each other, where both caused a significant (P<0.05) reduction in urea serum level in diabetic rabbits, as compared to the diabetic-control (table 6). Generally, DM is caused impairment of renal function but GH 300mg/kg and GHE (equivalent to 300mg/kg of GH) decreased both urea and creatinine serum levels probably by enhancing the renal function (Yassin and Mwafy, 2007; Daisy *et al.*, 2009).

Oxidative stress has been indicated one of the major causative factors which could induce a number of chronic and degenerative diseases like *Diabetes mellitus*, ageing, atherosclerosis, ischemic heart diseases, immunosuppressant, neoplastic disorders and neurodegenerative diseases. It is widely believed that chronic diseases like DM can induce the increase in oxidative stress (Veeru *et al.*, 2009). An increase in oxygen free radical production has been reported in diabetes. The compromised natural antioxidant mechanisms culminate the increase in oxidative stress (Luo *et al.*, 2004). Hence glycation of tissue proteins can lead to the high glucose serum level. Glycation along with glucose auto-oxidation can generate hydrogen peroxides, hydroxyl radicals and protein-reactive ketoaldehydes. Hyperglycemia can also lead to glycation of the lipoproteins, lipid peroxidation, superoxide production and oxidative DNA damage. Naturally antioxidants protect against free radical-induced damages (Luo *et al.*, 2004). Phenols and polyphenols like flavonoids, are widely distributed in food and food-products obtained from plant sources. These substances have been reported to have high level of antioxidant potentials. Further they can inactivate lipid free radicals and can prevent the conversion of hydro-peroxides into free radicals (Nahak and Sahu, 2010).

Plants are the rich source of natural antioxidants which could be useful in the treatments of chronic ailments. They are also of great interest to manufacture the food at industrial scale (Sweta and Seemi, 2012). Phytochemical screening (table 10) of *Gossypium herbaceum* L seed powder and its extracts revealed the presence of alkaloids, flavonoids, phenolic compounds, coumarins, steroids, saponins. Plants contains a number of free radical scavengers like phenolic compounds, flavonoids, lignins, vitamins, terpenoids, tannins, saponins, coumarins, quinones, amines, alkaloids and betalains. Plant phenolics including flavonoids and tannins have been reported to be a major group of compounds with great antioxidants potential (Darvhekar *et al.*, 2013).

Moreover, literature indicated clearly that plant sourced flavonoids and tannins had to have antidiabetic activity (Sharma *et al.*, 2010b; Velmurugan and Bhargava, 2014). Swanston-Flatt *et al*. (1990) reported the anti-hyperglycemic activity of *Allium sativum* due to the presence of flavonoids and sulfur containing compounds. Saponins have also been reported to have antidiabetic and antioxidant potential. Phyto-constituents such as alkaloids, flavonoids, tannins, terpenoids and saponins have been indicated to be active anti-hyperglycemic agents (Atchibri *et al.*, 2010). Hence the presence of flavonoids, tannins and saponins in *Gossypium herbaceum* L powdered seed and its extracts, may be responsible for antioxidant and antidiabetic activities. Yield variation among aqueous and ethanol extracts of GH may be due to water verses ethanol difference in polarity. Ethanol is considered an effective solvent to extract phenolic compounds (Siddhuraju and Becker, 2003). Therefore, yield of ethanol extract of GH was higher than that of aqueous extract of GH (Nahak and Sahu, 2010).

Furthermore, histological study; architecture of the islet of Langerhans in the pancreas of diabetic rabbits exhibited marked degeneration of β-cells as compared with the normal pancreas (fig. 2-7). The islets of diabetic rabbits are partly hylinised with damaged cell walls along with reduced number of islet cells and vacuolations, many hydropic, necrotic cells with pyknotic and hyperchromatic nuclei. The observations are comparable with the findings of Ahmad *et al.*, (2010). Treatment with GH 300mg/kg b.w. (fig. 4) and GHE (fig. 5) markedly succeeded to amend the disrupted islets of Langerhans of diabetic rabbits. The islet architecture is more organized with improved integrity; the islets appeared non-vacuolated, intact and completely restored (fig. 4 and 5). Similar results were observed by Xiu *et al.*, (2001). The architecture of islets treated with, GHA (fig. 6) and Glimepiride (fig. 7) reflected partial restoration of β-cells as compared to the diabetic-control (fig. 3). Data obtained (table 9) exhibited the dose dependent renewal and regeneration of β-cells where complete restoration of β-cells is evident at higher dose level *i.e.* 300mg/kg GH. These findings are in accord with the report of Gohil *et al.*, (2010).

CONCLUSION

It can be concluded that the prevention and restoration of islet β-cells in the presence of Alloxan induced destruction may be primary cause of recovery of Alloxan diabetic rabbits. The restoration of β-cells in diabetic treated rabbits corroborates the anti-oxidant activity in treated animals. Thus the results of present study clearly suggest that GH and GHE (equivalent to 300mg/kg of GH) had anti-hyperglycemic and anti-hyperlipidemic effects along with antioxidant activity in Alloxan diabetic rabbits. The GH and GHE remained highly effective for the management of associated complications with *Diabetes mellitus i.e.* hypercholesterolemia, hyper-triglyceridemia and renal function impairment. Therefore, *Gossypium herbaceum* L seed and its ethanol extract might possess therapeutic potential against *Diabetic mellitus*, as well as the development and progression of its complications.

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