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Research Article

Hypoglycemic and Antihyperglycemic Effects of Different Extracts and Combinations of *Withania coagulans Dunal* and *Acacia arabica Lamk* in Normal and Alloxan Induced Diabetic Rats

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ABSTRACT: **Introduction:** *Acacia arabica* and *Withania coagulans* commonly known as babool and paneer phool respectively are used in traditional Indian medicine for treatment of diabetes mellitus. **Methods:** The hypoglycemic effect of aqueous extracts (hot and cold water) and hydroalcoholic extract of *Acacia arabica* and *Withania coagulans* was investigated. Oral administration of a cold water extract of *Acacia arabica* bark, and a hydroalcoholic extract of *Withania coagulans* to diabetic and normal rats at a dose of 400 mg/kg body weight resulted in a significant reduction of blood glucose, cholesterol and triglycerides in alloxan induced diabetic rats. **Results:** Phytochemical investigation found that saponins, flavonoids, alkaloids and tannins were present in the *Withania coagulans* extracts, and phenolic compounds were presents in A*cacia arabica* extracts. The hydroalcoholic extract of *Withania coagulans,* and the cold water extract of A*cacia arabica* were found to reduce the blood-glucose level to normal level within seven days. Promising effects were observed when a combination of both extracts was administered to the test animals. Histological studies of the β-cells indicate that the extracts affect pancreatic cells. **Conclusions:** The hydroalcoholic extract of *Withania coagulans* and the cold aqueous extract of *Withania coagulans* exhibited antihyperglycemic activities in alloxan-induced diabetic rats. Combination of both plants in the ratio 400+400 mg/kg shows higher antihyperglycemic activities. Regereneration of β-cells, is seen in combinations of extract of both the plants.

KEYWORDS: *Withania coagulans*, diabetes mellitus, *Acacia arabica*, combinations.

INTRODUCTION

Diabetes mellitus is caused by an absolute or relative lack of insulin that, among other consequences, leads to an increase in plasma glucose concentration. In type I insulin-dependent diabetes mellitus [IDDM], previously called juvenile diabetes, there is an absolute lack of insulin. The condition is caused by a lesion in the beta cells of the pancreas. As the number of people with diabetes multiplies worldwide continues to increase, it is projected to become one of the world's main disasters. The regions with greatest potential for increased rates of DM are Asia and Africa, where DM rates could rise to two to three-fold the present rates.

Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations.[1] The phytochemical investigation in this study showed the presence of saponins, alkaloids, flavonoids and tannins. Hyperglycemia results in the generation of free radicals who can exhaust antioxidant defenses, leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to lipid peroxidation.[2,3] Flavonoids are one of the most numerous and widespread groups of phenolics in higher plants.[4-8] Some of them, due to their phenolic structure, are known to be involved in the healing process of free radical-mediated diseases, including diabetes.[5] Some are reported to be hypoglycemic in some literature.

Withania coagulans Dunal belongs to the family Solanaceae and is reported to have a number of pharmacological activity's, i.e. hepatoprotective and anti-inflammatory activity,[9] antifungal and antibacterial activity[10] and hypoglycemic activity[11] *Withania coagulans* is rich in steroidal lactones, which are known as withanolides. One of the characteristic features of the plants that produce withanolides

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is their extraordinary ability to introduce oxygen functions at almost every position of the carbocyclic skeleton and side chain. Previous phytochemical examination of the whole plant resulted in the isolation of 25 compounds, including 24 withanolides and one dimeric lignan, bispicropodophyllin glucoside.[12]

Acacia arabica belongs to family Mimosaceae and is reported for *In vitro* antibacterial activity[13] antimicrobial and immunomodulatory activities. Previous studies of the bark of *Acacia nilotica* show the presence of following chemical constituent's gallic acid, catechin 5-*O*-gallate, galloylated derivatives of catechin 5-O-gallate, the peltogynoids acanilol A ($C_{19}H_{16}O_7$) and B ($C_{18}H_{14}O_7$) diterpene niloticane ($C_{20}H_{30}O_3$), new compound gallocatechin 5-*O*-gallate in addition to methyl gallate, gallic acid, catechin, catechin 5-O-gallate, 1-*O*-galloyl-β-D-glucose 1,6-di-*O*-galloyl-β-D-glucose and digallic acid.

Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive antidiabetic principles.[14,15] Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats.[16] Phenolics have been found to be effective antihyperglycemic agents.[17] In the present study, hypoglycemic activity of different extracts of *Withania coagulans* and *Acacia arabica* and combinations of both were observed in alloxan induced diabetic albino rats.

MATERIAL AND METHOD

Plant material

Acacia arabica bark was collected from the village Garhpehra near Sagar (M.P.), India. Dried *Withania coagulans* fruits were purchased from a local market and identified by chief botanist of Dr H.S.Gour University Sagar (Dr. Pradeep Tiwari). A voucher specimen was deposited in the Herbarium, Botany Department, University Sagar. Accession numbers of the herbs are *Withania coagulans*: Bot/H/3362, *Acacia arabica*: Bot/H/2698

Preparation of extracts

Hydroalcoholic extract

The dried fruits of *Withania coagulans* and *Acacia arabica* bark were finely powdered and extracted by hot percolation method using Soxhlet apparatus. The solvent used was 50% methanol. After extraction the extract was dried in a water bath at a temp 35-40 °c (yield 25.54% w/w).

Hot water extract

The dried fruits *Withania coagulans* and *Acacia arabica* were finely powdered and extracted by boiling with water for 2 hr. After extraction the extract was dried in a water bath at a temp 35-40 °c (yield 9.926% w/w).

Cold water extract

The dried fruits *Withania coagulans* and *Acacia arabica* were finely powdered and extracted by macerating with water for five days. After extraction the extract was dried in a desiccator. (Yield 14.028% w/w).

Animals

Albino Wistar rats (120-150 g) 60 days old of either sex were obtained from CRC BMCP Mandsaur (M.P). Before and during the experiment rats were fed with standard diet (Lipton, India Ltd). After randomization into various groups, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum.

Sample collection

Blood samples were collected from tail vein and the blood glucose content was estimated using electronic glucometer (Smart Care, Taiwan).

Preliminary oral LD50 determination

Preliminary oral LD50 dose of *Withania coagulance* hydroalcoholic extract(WCHAE),*Withania coagulance* Hot water extract extract (WCHWE),*Withania coagulance* cold water extract extract (WCCWE), *Acacia arabica* hydroalcoholic extract(AAHAE), *Acacia arabica* Hot water extract extract(AAHWE), *Acacia arabica* cold water extract extract (AACWE) in rats were determine according to the OECD guideline.

Experimental design

All the animals were divided into the six groups with five animals in each group:

Group I : Normal control.
Group II : Diabetic control (alloxan treated without other treatment).
Group III : Diabetic rat treated with Glibenclamide.
Group IV : Treated with WCHAE (with and without treated alloxan).
Group V : Treated with WCHWE (with and without treated alloxan).
Group VI : Treated with WCCWE (with and without treated alloxan).
Group VII : Treated with AAHAE (with and without treated alloxan).
Group VIII : Treated with AAHWE (with and without treated alloxan).
Group IX : Treated with AACWE (with and without treated alloxan).
Group X : Treated with WCHAE+AACWE (400 mg/kg + 400 mg/kg)

Group XI : Treated with WCHAE+AACWE (400 mg/kg + 200 mg/kg)
Group XI1 : Treated with WCHAE+AACWE (200 mg/kg + 400 mg/kg)

Assessment of extracts and combinations on glucose level of normal animals

Rats were divided in different groups and their normal glucose levels were determined with the help of glucometer. Different doses of the extracts were administered with the help of oral feeding tube and the glucose level was determined at different time interval i.e.60, 120.150,180 mins post treatment.

Assessment of extracts and combinations on alloxan-induced diabetic animals

Rats were induced to become diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg).[18] Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2 ml saline just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection. Fasting blood glucose estimation and body weight measurement were recorded on day 1 and 7 of the study. On day 7, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated.[19] Serum was separated and analyzed for serum cholesterol,[20] serum triglycerides and serum creatinine by enzymatic DHBS method.[21] The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution and processed for histological examination.

Statistical analysis

All the values of fasting blood sugar and biochemical estimations were expressed as mean ± standard error of means (S.E.M.) and analyzed by ANOVA and post hoc Dunnett's t-test. Differences between groups were considered significant at $P < 0.05$ levels.

RESULTS

Acute toxicity

The LD_{50} dose of all the extract was found to be 400 mg/kg, where as in case of AAHAE the LD50 dose was found to be 196 mg/kg due to some toxicity the detail study in this relation is under progress.

Acacia arabica and *Withania coagulans* extract studies

Alloxan is cytotoxic to the pancreatic β-cells thus it is an effective diabetes-induction agent. It has previously been widely used to induce diabetes mellitus in experimental animal models, allowing investigation of hypoglycemic agents in the treatment of diabetes.[22,23] Alloxan injection consistently produced symptoms characteristic of diabetes mellitus including hyperglycemia, decreased insulin levels, polyurea and weight loss[33]. In our approach, we demonstrated the efficacy of Alloxan through the glibenclamide studies in diabetic rats as well as in normal hyperglycemic rats[8]. In the present study, the hypoglycemic activity of cold water extracts, hot water extracts and hydroalcoholic extracts from *Acacia arabica bark* and *Withania coagulans* were evaluated in normal and alloxan-induced diabetic rats. A single oral administration with a combination of all the three extracts from *Acacia arabica* bark and *Withania coagulans* caused a significant decrease in serum glucose levels in normal rats with dose 400 mg/kg b/w for AACWE and AAHWE whereas in AAHAE dose is 196 mg/kg b.w. (Table 1). Moreover, these doses of the extract from *Acacia arabica* bark and *Withania coagulans* produced the maximum glucose lowering in diabetic rats serum (Table 2). A significant time-dependent hypoglycemic effect was shown throughout

Table 1: Effect of different extracts of *Withania coagulans* and *Acacia arabica* on blood glucose level of normal rats

Group No	Treatments	Dose mg/kg p.o	0 min	60 min	120 min	180 min
I	Normal control	5 ml	84.00 ± 2.14	83.80 ± 1.96	83.00 ± 1.76	82.20 ± 1.68
II	Standard drug	10	88.00 ± 0.71	72.40 ± 0.92**	65.80 ± 1.28**	67.21 ± 0.86**
IV	WCHAE.	400	84.40 ± 1.50	74.00 ± 0.70**	71.20 ± 1.39**	67.40 ± 1.53**
V	WCHWE.	400	82.6 ± 2.21**	83.2 ± 2.17**	81.4 ± 3.50**	78.6 ± 3.45**
VI	WCCWE.	400	84.60 ± 1.63**	86.40 ± 1.69**	85.6 ± 1.03**	83.00 ± 1.14**
VII	AAHAE.	196	88.6 ± 0.88	82.61 ± 1.54*	79.0 ± 2.08**	80 ± 0.54**
VIII	AAHWE.	400	87.12 ± 1.52	78.33 ± 0.33**	65.67 ± 0.88**	66.0 ± 0.57**
IX	AACWE.	400	87.00 ± 1.14	66.81 ± 2.70**	60.60 ± 2.54**	61.20 ± 1.81**
X	Com 1	400 + 400	86.1 ± 1.67	66.7 ± 2.88**	54.8 ± 1.85**	45 ± 2.96**
XI	Com 2	400 + 200	84.4 ± 1.69	69.0 ± 3.63**	56.61 ± 2.82**	48.2 ± 2.51**
XII	Com 3	200 + 400	83.79 ± 1.24	71 ± 2.86**	57.80 ± 2.57**	54.4 ± 2.83**

Values are given in average body weight (g) ± SEM for groups of five animals each. Vehicle (Tween 80). Diabetic control 150mg/kg b. w. dose. (Alloxan)
*P < 0.05 as compared to vehicle control. **P<0.01 as compared to normal. WCHAE. *Withania coagulance* hydroalcoholic extract; WCHWE. *Withania coagulance* Hot water extract extract; WCCWE *Withania coagulance* cold water extract extract; AAHAE. . *Acacia arabica* hydroalcoholic extract; AAHWE. *Acacia arabica* Hot water extract extract; AACWE. *Acacia arabica* cold water extract extract; Com 1. *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract; Com 2 *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract; Com 3 *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract.

the period studied (Table 3). Based on the hypoglycemic effect in normal and diabetic rats, these results reinforce the hypothesis that the hypoglycemic mechanism involves an insulin-like effect, possibly through peripheral glucose consumption.[24,25,26] Although the cold water extract from *Acacia arabica* bark and hydroalcoholic extract of *Withania coagulans* displayed a higher significant hypoglycemic effect in normal rats, the main mechanism by which *Acacia Arabica* and *Withania coagulans* brings about its hypoglycemic action probably is by stimulating peripheral glucose consumption. Whereas it is assuming that the *Acacia arabica* cold water extract exert its action similar to glibenclamide. A number of other plants have also been reported to have hypoglycemic effects[27,28]. From the studies with the tested plant extract, the chronic effect antihyperglycemic activity of AACWE and WCHAE was demonstrated at a dose of 400 mg/kg (Table 3). Consequently, this dosage was considered as a quantitative basis to study in severe alloxan-diabetic rats or in normal animals.

Combinations of active extract of *Withania coagulans* and *Acacia arabica*

The hypoglycemic activity of different combinations of active extracts of *Withania coagulans* fruit and *Acacia arabica* bark (i.e. AACWE and WCHAE) was evaluated in normal and alloxan-induced diabetic rats. A single oral administration with all the three extracts caused a significant decrease in serum glucose levels in normal rats with dose 400mg/kg + 400mg/kg b.w all extracts studied (Table 1). Moreover, these doses of the extracts produced the maximum glucose lowering in diabetic rats serum (Table 2), a significant time-dependent hypoglycemic effect was shown throughout the period studied (Table 3). From the studies with the tested plant extract, the optimal antihypeglycemic activity was demonstrated at a dose of 400 +400 mg/kg.

In histological slides the β-cells are completely destroyed in the diabetic group animal (Figure 1). In contrast, the animals which are given with combination of extract have shown regeneration of the β-cells. No such regeneration was seen in the rats treated with single *Withania coagulans* or *Acacia arabica* extracts, no regeneration of the β-cells are seen.

DISCUSSION

The pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose.

Table 2: Effect of different extracts of *Withania coagulans* and *Acacia arabica* on blood glucose level of diabetic rats

Group No	Treatments	Dose mg/kg p.o	0 min	60 min	120 min	180 min
I	Normal control	5 ml	84.00 ± 2.14	83.80 ± 1.96	83.00 ± 1.76	82.20 ± 1.68
II	Diabetic control	5 ml	215.20 ± 4.18	217.00 ± 3.61	216.80 ± 3.45	217.60 ± 4.54
III	Standard drug	10	280.41 ± 3.81	214.2. ± 4.9**	174.2 ± 4.41**	173.0 ± 3.22**
IV	WCHAE.	400	283.80 ± 1.59	242.00 ± 1.26**	233.07 ± 1.62**	231.40 ± 1.36**
V	WCHWE.	400	233.60 ± 7.5	231.00 ± 6.59**	230.20 ± 6.88**	227.80 ± 5.57**
VI	WCCWE.	400	226.00 ± 7.71	224.80 ± 5.57**	228.60 ± 6.03**	216.61 ± 4.65**
VII	AAHAE	196	227. ± 5.41	205.81 ± 5.67*	187.40 ± 4.49**	185.6 ± 3.23**
VIII	AAHWE.	400	224.6 ± 3.28	197.2 ± 6.93**	180.2 ± 5.39**	179 ± 5.76**
IX	AACWE.	400	218.8 ± 3.41	201.0 ± 5.9*	174.80 ± 2.87**	169.20 ± 4.22**
X	Com 1	400 + 400	289.2 ± 1.74	225.4 ± 9.17**	189.60 ± 3.04**	184.2 ± 5.64**
XI	Com 2.	400 + 200	292 ± 1.92	232.61 ± 8.34**	197.40 ± 2.94**	193 ± 1.78**
XII	Com 3.	200 + 400	283.4 ± 9.70	241.6 ± 8.81**	205.8 ± 4.18**	208.4 ± 2.27**

Values are given in average body weight (g) ± SEM for groups of five animals each. Vehicle (Tween 80). Diabetic control 150mg/kg b. w. dose. (Alloxan).
*P < 0.05 as compared to vehicle control. **P<0.01 as compared to normal. WCHAE. *Withania coagulance* hydroalcoholic extract; WCHWE. *Withania coagulance* Hot water extract extract; WCCWE *Withania coagulance* cold water extract extract; AAHAE. . *Acacia arabica* hydroalcoholic extract; AAHWE. *Acacia arabica* Hot water extract extract; AACWE. *Acacia arabica* cold water extract extract; Com 1. *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract; Com 2 *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract; Com 3 *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract.

Table 3: Effect of WCHAE, AACWE and COM 1 on blood glucose level of alloxan induced diabetic rats (chronic effect)

Group No	Treatments	Dose mg/kg p.o	1st Day	3rd Day	5th Day	7th Day
I	Normal control	5 ml	84.00 ± 2.14	85.12 ± 2.16	83.10 ± 3.14	85.1 ± 1.34
II	Diabetic control	10	278.20 ± 4.18	280.15 ± 4.15	281.36 ± 2.56	280.14 ± 2.24
III	WCHAE	400	283.80 ± 1.59	222.61 ± 1.48**	101.23 ± 2.16**	99.22 ± 1.35**
IV	AACWE.	400	225.75 ± 1.59	179.23 ± 1.82**	134.53 ± 1.39**	87 ± 1.56**
V	COM 1.	400 + 400	343.8 ± 1.53	171.04 ± 1.43	114.31 ± 2.48	87.4 ± 0.812**

Values are given in average body weight (g) ± SEM for groups of five animals each. Vehicle (Tween 80). Diabetic control 150mg/kg b. w. dose. (Alloxan).
*P < 0.05 as compared to vehicle control. **P<0.01 as compared to normal. WCHAE. *Withania coagulance* hydroalcoholic extract; AACWE. *Acacia arabica* cold water extract extract; Com 1. *Withania coagulance* hydroalcoholic extract and *Acacia arabica* cold water extract extract.

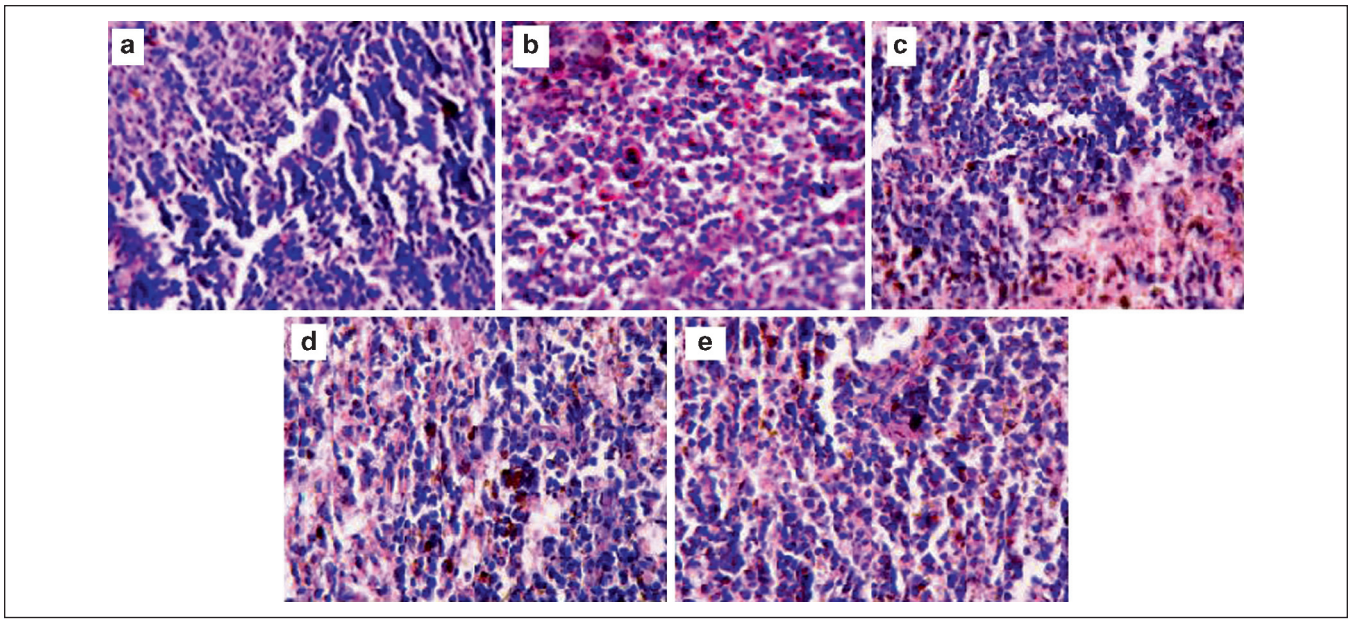

Figure 1: A low magnification image of pancreas (H and E stain). (a) Diabetic pancreas: - pancreatic artery and vein is completely destroyed Irregular β-cells are seen in section. (b). Normal pancreas: systematically arranged β-cells are seen in the Section. (c) *Withania coagulans* treated diabetic pancreas: No regeneration of β-cells are seen in section. (d) *Acacia arabica* treated diabetic pancreas: Some regeneration of β-cells are seen in section but the quantity of live β-cells are very less. (e) Combination treated diabetic pancreas: Regeneration of β-cells are seen in section.

Insulin is secreted[29]. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative[30] and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus Von Liebig. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans[31].

According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on β-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption[32-33]. In light of the results, our study indicates that Acacia Arabica and Withania coagulans extracts have good antidiabetic activity. The number of functionally intact beta-cells in the islet organ is of decisive importance for the development course and outcome of diabetes. The renewal of beta-cells in diabetes has been studied in several animal models. The total beta-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet beta-cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug[34]. In our studies, the damage of pancreas in alloxan-treated diabetic control rats (Fig. 1a) and regeneration of beta-cells by *Acacia arabica* and combination of extracts (Figure 1c and 1d) was observed.

CONCLUSION

The hydroalcoholic extract of *Withania coagulans* and the cold aqueous extract of *Withania coagulans* exhibited antihyperglycemic activities in alloxan-induced diabetic rats. Combination of both plants in the ratio 400 + 400 mg/kg shows higher antihperglycemic activities than for either extract alone.

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