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Protective effects of hydro-ethanolic extract of *Ricinus communis* flowers against oxidative stress in diabetes induced swiss albino mice

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ABSTRACT:

Hydroethanolic extract of *Ricinus communis* flowers was studied for treating diabetes induced oxidative stress in adult male swiss albino mice. The experimental animals were divided into four groups of seven mice each viz., Normal Control (NC), Diabetic Control (DC), Diabetic + *R. communis* Flower extract treated (FT) and Diabetic + Glibenclamide treated (GT). The groups DC, FT and GT were given a single intraperitoneal injection of Alloxan monohydrate (150 mg/kg body weight). Alloxan administration induced destruction of beta cells, hampering production of insulin and aggravating blood glucose levels. It also resulted in over-production of Reactive Oxygen Species (ROS) and thus oxidative stress. Groups NC and DC received normal saline while FT was given *Ricinus Communis Flower Extract* (RCFE) at 300 mg/kg body weight and GT was treated with Glibenclamide, for a period of 45 days. Fasting Blood Glucose (FBG) levels were observed at regular intervals. After the treatment period, the liver, kidneys and pancreas of the experimental animals were estimated for their antioxidative status. The antioxidant markers such as Catalase (CAT), Glutathione peroxidase (GPx), reduced Glutathione (GSH) and Superoxide dismutase (SOD) saw a variation from their normal values. Treatment of Alloxan induced diabetic mice with RCFE for 45 days lead to a significant *($P<0.05$)* normalization of FBG levels and antioxidant parameters. Hence, indicating its protective effects against diabetes induced oxidative stress.

Keywords: *Ricinus communis*, RCFE, Diabetes, Oxidative stress, Reactive Oxygen Species, Antioxidant.

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INTRODUCTION:

Diabetes is known as a metabolic disorder of the endocrine system and is posing a major health problem all over the world. In diabetics, lack of glucose metabolism results in an increased level of glucose in the blood. Increasing evidences have confirmed that oxidative stress majorly contributes in the pathogenesis of diabetes[1]. The over-production of ROS, specially the superoxide free radicals, results through the electron transport chain in the mitochondria[2]. Thus, oxidative stress biomarkers, such as superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, vitamins, lipid peroxidation, nitrites, non-enzymatic glycosylated proteins etc. show variations as compared to their normal values which may lead to complications[1].

Commercially available hypoglycemic drugs may have side effects. Therefore, there is always a need for developing new oral anti-hyperglycemic drugs which help not only in controlling diabetes but also related oxidative stress. Plants have long history of use and better patient tolerance as well as acceptance. However, few have been scientifically evaluated for their protective ability against diabetes related oxidative stress. *Ricinus communis*, popularly known as 'Castor Oil Plant' is a robust perennial shrub of Euphorbiaceae family. It is a short heighted tree, spread widely across the tropics and high temperature areas of the world. Its oil and other components have been known to exhibit numerous biological activities, especially antimicrobial activity. Owing to their potential use, it has often been the subject of study in the chemical and pharmaceutical industries[3].

The plant is used in treating several aliments as: an antidote, a bactericide, for arthritis, asthma, cholera, convulsions, dermatitis, epilepsy, inflammation, tuberculosis and wounds. The oil and seeds are used for the treatment of warts and cold tumors. Castor-oil is used as a cathartic and possesses labor-inducing properties[4]. Leaves are applied externally to the head to relieve headache and as a poultice for boils[5]. Antioxidant activity of this plant has been studied by some *in vitro* assays and the plant has also been reported for being anti-diabetic[6].

Plenty of bioactive compounds such as glycosides, terpenoids, flavonoids, tannins etc. have been found in flowers of the plant. However, the anti-hyperglycemic and anti-oxidative potential of flower extract of *R. communis* has not been worked out. With this perspective, the present study, evaluates the effect of 45 day treatment of RCFE on diabetes and related oxidative stress induced by Alloxan in male swiss albino mice.

MATERIALS AND METHODS:

Chemicals

All the chemicals brought in use for this study were of analytical grade and were purchased from HIMEDIA (India), SRL (India), CDH (India), SD Fine (Mumbai, India), Qualigens (India/ Germany) and Lobachemie Pvt. Ltd.

Preparation of extract

Flowers of *R. communis* were collected from the plants available at Agricultural Research Institute, Mandor (Jodhpur, Rajasthan, India). They were identified taxonomically by a botanical expert. Dried flowers were reduced into a coarse powder using an electric grinder. The powder was then subjected to soxhlet extraction using 50% hydro-ethanol. The extract so obtained was then dried in a vacuum rotatory evaporator under reduced pressure at 60±1°C. The concentrated extract was kept in a hot air oven at 40-45°C till it dried to a semisolid mass. As compared to crude material the yield of extract was 6.3% w/w. For treating the experimental animals, the doze was prepared by suspending the extract in 20% Tween 20 (prepared in normal saline)

Animal care and monitoring

Healthy (weighing 25-35 gm), 6-8 months old, male Swiss albino mice (*Mus musculus*) were procured from C.C.S. Haryana Agricultural University (Hissar, India). The experimental animals were kept under standard laboratory conditions: Light (12:12 h L: D cycle), Temperature (23 ± 2°C) and Relative Humidity (55 ± 5%). They were fed standard rat pellet feed and tap water *ad libitum.* The treatment and maintenance of all the animals was in accord with the principles prescribed by the Institutional Animal Ethics Committee, constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Introduction of diabetes and treatment

For this study, experimental animals were segregated into following groups of seven mice each:

NC	DC	GT	FT
Normal Control	Diabetic Control	Glibenclamide Treated	Diabetic + RCFE Treated

Diabetes was induced by a single intra-peritonial injection of Alloxan monohydrate (150 mg/kg body weight), freshly dissolved in normal saline in mice of groups DC, GT and FT after overnight fasting[7]. Subsequently, free access to food and 10% glucose solution was provided to the mice to counter the hypoglycemic shock after Alloxan injection. After one week of Alloxan injection, the Fasting Blood Glucose (FBG) concentration was observed by using Dr, Morepen's One Touch Ultra glucometer and compatible blood glucose strips by Johnson & Johnson Company, USA[8]. Mice with FBG level greater than 140 mg/dl were considered to be diabetic[9] and were therefore

selected for treatment. The drug (10 mg/kg body wt) and the flower extract (300 mg/kg body wt) were administered orally, once in a day, for 45 days.

Estimation of FBG levels

FBG levels of the four experimental groups were noted by means of a glucometer (DrMorepen One touch glucometer) at regular intervals of the experiment by collecting a drop of blood from the tail vein of each animal. FBG levels were expressed in mg/dl.

Estimation of antioxidants

After the 45 day experiment, the animals were sacrificed by cervical dislocation. The organs: Liver, pancreas and kidney were collected, washed off the adhering tissues and blood with ice-cold normal saline solution (0.9%). All the organs were weighed only after drying them. Tissues were then homogenized in 0.2 M tris-HCl (10 ml of tris-HCl for every 1 g of tissue). The homogenates were subjected to centrifugation at 10,000 rpm for 20 minutes at 4°C. The supernatants collected were used for estimation Superoxide Dismutase (SOD) [10], Catalase (CAT) [11], Glutathione Peroxidase (GSH-Px) [12], reduced Glutathione (GSH) [13] and Thiobarbituric acid reactive substances (TBARS) [14].

Statistical Analysis

Results are expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc multiple comparison test using SPSS (version 16.0) and student's't'-test using SigmaPlot (version 8.0). The values of $P<0.05$ were considered as statistically significant.

Ethical clearance:

The experiments on animals were designed in accord with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The approval of the Institutional Animal Ethics Committee was taken for the use of Swiss Albino mice as an animal model for this study.

RESULTS AND DISCUSSION:

Effect on fasting blood glucose level:

Alloxan injection to DC, FT and GT groups resulted in significant ($P<0.05$) aggravation of blood glucose levels or hyperglycemia unlike the normal control group, NC. Hyperglycemia is the major symptom which leads to diabetic complications[15]. Treatment with Flower extract helped in significantly lowering the glucose level by 36.61% (i.e. from 180.285± 19.4 mg/dl to 114.28 ± 10.87 mg/dl) to normalization. The reduction in the FBG levels by RCFE can be compared to the

FBG lowering effect of glibenclamide, which produced 53.54% reduction (from 206 ± 35.37 mg/dl to 95.7 ± 18.1 mg/dl) in blood glucose level of group GT after 45 days of dosage (Table1).The study revealed that RCFE significantly decreased the FBG levels in diabetic mice. The fact that this extract has helped in lowering the increased blood glucose level, can correspond to the presence of some constituents which might have insulin like activity, and could have helped in reducing the blood glucose level despite of disturbed insulin secretion. However, the possibility of synergistic effect of insulin stimulating action of other constituents in the extract cannot be ruled out.

Table 1: Effect of 45 day treatment of *R. communis* flower extract on FBG levels

Day	NC	DC	FT	GT
Before induction	106.14 ± 13.18	102 ± 3.36	110 ± 16.5	99 ± 17.54
0	106.14 ± 13.18 b	185.57 ± 13.91 a	180.285± 19.4 a	206 ± 35.37 a
45	103.71 ± 9.8 b	163.71 ± 16.96 a	114.28 ± 10.87 b c	95.7 ± 18.1 b c

Values are mean ± SEM of 7 observations.

Before induction (Basal values); Student's't'-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to basal values; c: significant ($P<0.05$) difference compared to values obtained after alloxan injection

Effect on various antioxidant markers:

Alloxan is a drug that causes β-cell cytotoxicity. Inside the body, it instinctively and affinitively gets deposited only in the pancreatic β-cells and stimulates their apoptosis and destruction by over-production of Reactive Oxygen Species (ROS) superoxide radicals and peroxides[15, 16]. β-cell death consecutively leads to insulin deficiency which further causes hyperglycemia[17], which leads to diabetic complications[15]. It is proven that an overload in the mitochondrial glucose causes an increased transfer of electrons to oxygen and thus generation of oxygen free radicals. This as a consequence stimulates the cellular reactions leading to diabetic complications along with hyperglycemia[16].Enhanced glucose metabolism via the Polyol (sorbitol) pathway is accompanied with hyperglycemia, which also leads to further over-production of Oxygen free radicals[17]. Significantly more oxidative stress is reported in patients with diabetes than in healthy individuals. It was observed that injecting Alloxan to mice lead to a significant (P<0.05) decrement in the antioxidant enzyme markers such as GSH, CAT, GPx and SOD in all the three studied tissues-liver, kidney and pancreas (Table 2). However, the decrease in GSH level was not significant. 45 days of Glibenclamide treatment could not restore the enzymatic antioxidant level, as significantly (P<0.05) lower values of enzyme activities can be seen under GT. Consequently, 45 days treatment with RCFE showed an overwhelming significant (P<0.05) increment in most of the cases in FT,

except for pancreatic GPx and SOD levels where the values were not normalized. The TBARS content saw a manifold increase after the induction of diabetes. Although, both the treatments with Glibenclamide and that with RCFE significantly lowered the TBARS level, the flower extract treatment almost normalized the TBARS content. Glutathione-S-Transferase (GST**)** found in various organs carries out many functions. One of its major roles is shielding the cells from per-oxidative damage[18, 19].

Table 2: Effect of 45 day treatment of *R. communis* flower extract on antioxidative status

Parameters		**NC**	**DC**	**FT**	**GT**
Hepatic	GSH	39.2 ± 5	12.3 ± 1.8 a	30.7± 7.23 a* bc	19.2 ± 1.65 a b*
	TBARS	34.2 ± 4.4	432.14 ± 16.9 a	58.47 ± 9.7 a*b c	234.51 ± 1.4 a b
	CAT	208.2 ± 9.43	136.3 ± 6.6 a	214.4 ± 9.84a*bc	150.5 ± 7.8a b*
	GPx	217.5 ± 12.7	122.7 ± 10.3 a	196.5 ± 23.7a*bc	148.4 ± 6.5a b*
	SOD	190.2 ± 15.4	140.7 ± 5.14 a	178.4 ± 9.43a*bc	132.6 ± 2.51a b*
Pancreatic	GSH	11.9 ± 4.4	7.8 ± .4 a*	9.6 ± 2.7 a*b*	7.5 ± 0.78 a b*
	TBARS	13.25 ± 1.28	102.24 ± 14.8 a	18.73 ± 1.4 a* bc	42.16 ± 2.53 a b
	CAT	214.3 ± 8.9	153.3 ± 2.85 a	224 ± 17.43a*bc	194.2 ± 9.5a* b
	GPx	172.1 ± 22.3	89.01 ± 9.75 a	128.4 ± 7.8 abc*	105.16 ± 20.2ab*
	SOD	220.3 ± 14.5	197.4 ± 27.9 a	181.1 ± 11.9ab*c	208.4 ± 15.9a b*
Renal	GSH	27.36 ± 8.6	8.7 ± 2.3 a	18.6 ± 1.28a*bc*	17.8 ± 3.4 a* b
	TBARS	63.75 ± 9.07	462.4 ± 13.98 a	47.83 ± 5.9 a*b c	207.8 ± 3.91 a b
	CAT	268 ± 2.64	146.4 ± 5.7 a	285 ± 4a*bc	196.5 ± 3.9a b
	GPx	167.8 ± 8.6	84.23 ± 14.6 a	178.67±13.6a*bc	96.2 ± 5.7 a b*
	SOD	198.5 ± 26.07	124.2 ± 12.17 a	129 ± 3.4a b*c*	113.8 ± 9.13a b*

Values are mean ± SEM of 7 observations.

GSH: mg/gm tissue, TBARS: nM TBARS/mg protein, SOD: Units/min/mg protein; Students' 't' test is significant at P<0.05. a: significant (P<0.05) difference, a*: insignificant (P<0.05) difference compared to NC, b: significant (P<0.05) difference, b*: insignificant (P<0.05) difference compared to DC, c: significant (P<0.05) difference, c*: insignificant (P<0.05) difference compared to GT.

Individuals suffering from diabetes show a reduced activity of GST in liver and kidney[20]. Significantly increased levels of GST in the RCFE treated group FT, therefore, proved the protective effect of the treatment over oxidative stress. Tissues of diabetes affected individuals are reported to be more vulnerable to lipid peroxidation[21]. Most of phyto-constituents act as radical scavengers, some of them cause reduction of the free radicals by lending hydrogen atoms, some of them play role as chain blocking agents in lipid peroxidation therefore, a significant decrease in lipid peroxidation (a marker of oxidative stress) was seen in tissues of the extract treated individuals. Besides, the antioxidant property of the extract was further stated by a significant

increase in the enzymatic antioxidants in the diabetics of group FT.SOD, CAT and GPx are enzymatic antioxidants. SOD catalyzes the dismutation of O_2 - to H_2O_2 and O_2[22, 23]. CAT is a main antioxidant defense protein that majorly catalyzes the decomposition of H_2O_2 to H_2O, GPx also shares this function[24]. Formation of ROS is also unavoidable in diabetic conditions[25]. The damage caused about by oxidative stress gets more severe, as the key antioxidant enzymes are lost consecutively, copper-zinc SOD[26] and CAT[27] are rendered inactive by glycation, resulting into a disturbance in the cell's reducto-oxidative environment[26]. Our study demonstrates a declination in the content of antioxidant marker enzymes such as CAT, GPx and SOD in various tissues of diabetic animals. However, the 45 day RCFE treatment motivated the activity of CAT, SOD and GPx to almost normal values in liver, pancreas and kidneys of experimental animals. Similar observations have also been reported by earlier studies by Hussain[28]. The observed anti-diabetic and antioxidative effect of RCFE was better than that of glibenclamide. Antioxidants are known to protect the β-cells which escaped destruction due to Alloxan, by their free radical scavenging action[29]. Restorative action of RCFE can be accredited to its phytoconstituents such as flavonoids, tannins, terpenoids and glycosides which act as antioxidants against induced cell damage and are capable of β-cell regeneration and thus lower the blood glucose concentration[30].

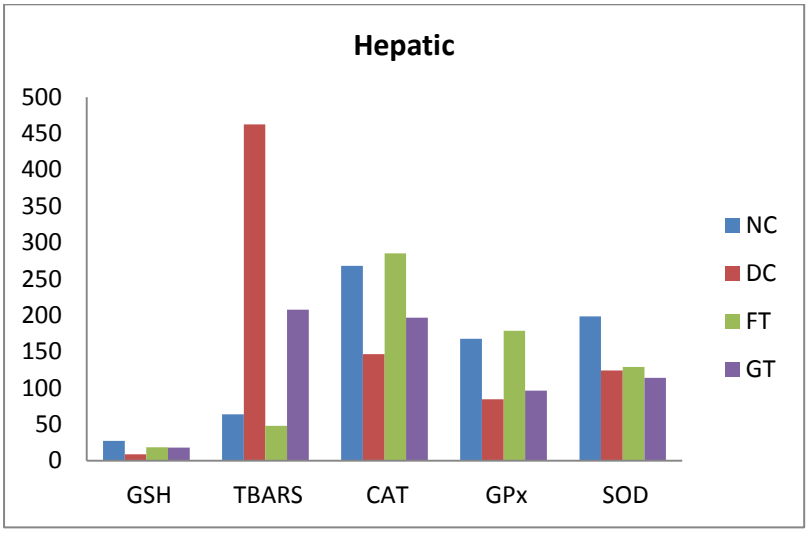

Figure 1: Oxidative stress management in the Liver

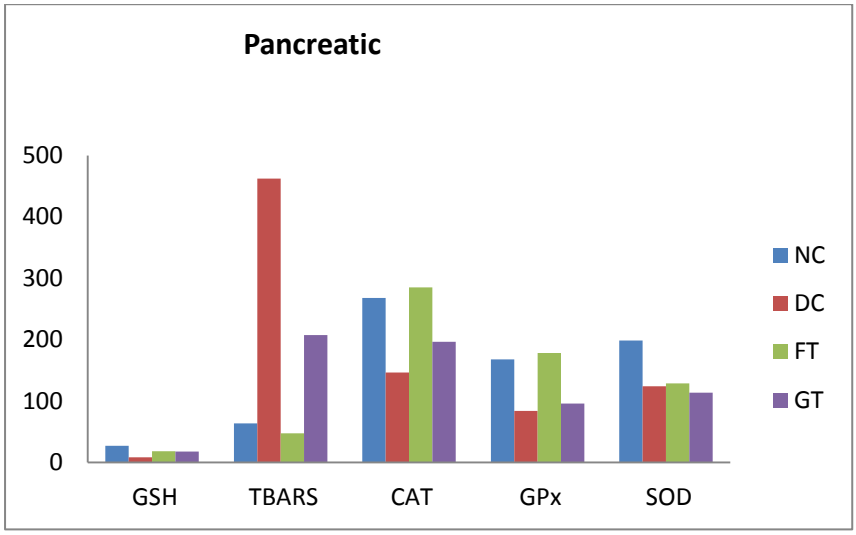

Figure 2: Oxidative stress management in the Pancreas

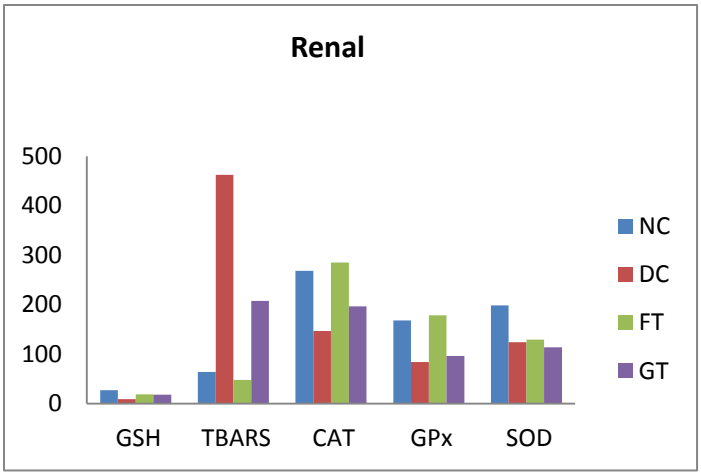

Figure 3: Oxidative stress management in the Kidney

GSH: mg/gm tissue, TBARS: nM TBARS/mg protein, CAT: µ moles H2O2 decomposed/min/mg protein, GPx: µg GSH consumed/min/mg protein, SOD: Units/min/mg protein; Student's'-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant ($P>0.05$) difference compared to NC; b: significant ($P<0.05$) difference, b*: insignificant ($P>0.05$) difference compared to DC; c: significant ($P<0.05$) difference, c*: insignificant ($P>0.05$) difference compared to GT.

CONCLUSION:

It can be concluded that the synergistic action of various phytoconstituents present in the flower extract of *R. communis* in contrast to the action of the purified compound of the standard drug was far more effective. The extract not only exhibits an antihyperglycemic activity, it also displays an optimistic antioxidative potential which is not shown in case of Glibenclamide treatment. The possible mechanisms of action of the extract are needed to be investigated. This work could be

further used to establish safety profiles and use of *R. communis* flower extract for the management of diabetes and oxidative stress in the clinic and thus developing new drugs from this plant.

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