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## HEPATOPROTECTIVE ACTIVITY AND OXIDATIVE STRESS OF THE AQUEOUS EXTRACT OF SEED FROM PINTO BEAN (*PHASEOLUS VULGARIS* L.) IN STREPTOZOTOCIN INDUCED ALBINO WISTAR RATS

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#### **Keywords:**

Exocrine gland, liver, Diabetes, Organic chemistry, Serum insulin

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**ABSTRACT:** The aim of the study was to evaluate the anti-diabetic and antioxidant effects of aqueous extract of *Phaseolus vulgaris* L. seeds in normal and streptozotocin (STZ) induced diabetic rats. Female Albino Wistar rats were divided into 6 groups of 6 rats; each was assigned into diabetic and non-diabetic groups. Diabetes was induced in rats by single intraperitoneal administration of STZ (55 mg/kg body weight). *Phaseolus vulgaris* L. extract at the doses of 100, 200, and 300 mg/kg body weight and glibenclamide (600 μg/kg b.w.) by intragastric tubation orally administered to both diabetic animals for a period of 30 days. After completion of experimental length, blood serum, liver, and exocrine gland were used for evaluating organic chemistry. Oral administration of *Phaseolus vulgaris* L. seeds significantly reduced elevated serum glucose, renal, liver function levels and significantly increased serum insulin and body weight as well as improved lipid profile due to diabetes.

**INTRODUCTION:** Diabetes mellitus (DM) is one of the common endocrine metabolic disorders with micro and macro vascular complications leading to mortality and morbidity all around the world <sup>1</sup>. The international diabetes Federation figured that during 2017 there are 451 million (age 18-99 years) people with polygenic disorder globally these figures were expected to increase to 693 million *via* 2045 <sup>2</sup> changes in lifestyle, consumption of energy-wealthy diets, and weight problems are some of the factors inflicting the upward push in the number of produced within the human system triggers oxidative harm by way of release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from activated neutrophil and macrophages.



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This over manufacturing results in damage of diverse organs leading to various illnesses like heart disease autism, cancer, diabetes, arthritis, Alzheimer's dementia, Parkinson's disease. cataracts and growing old <sup>3</sup>. Hyperglycemia in diabetes contributes to oxidative stress as evident from several research undertaken, it's far of tremendous significance of nutritional supplementation of antioxidants may also reduce the oxidative strain and protect tissues from ROS harm <sup>4</sup>.

Such supplementation may additionally have a shielding function and has been correlated with a lower within prevalence of numerous degenerative sicknesses, which includes diabetes and its complications <sup>5, 6</sup>. At present therapeutic options for diabetes are food regimen, exercising, oral hypoglycemic capsules, and insulin therapy. These tablets had been used as monotherapy or in different combos with a view to manipulating diabetic circumstances.

solvent for 16 hours and was concentrated under reduced pressure in a rotary evaporator at 60±10°C

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to yield the required quantity of crude extract. The extract was stored and used for further studies

**Phytochemical Screening:** Preliminary phytochemical analysis was carried for the presence of various phytoconstituents such as steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, terpenoids, tannins, and phenolic compounds as per standard methods <sup>13, 14</sup>.

Chemicals and Solvents: All the chemicals and solvents were of analytical grade and obtained from Fischer Inorganic and Aromatic Limited, Chennai, India Streptozotocin was purchased from SD Fine Chem. Limited, Mumbai, India.

**Experimental Animal:** Experiments were performed using sexually mature female albino Wistar rats weighing around 180-200 g. Rats were provided with standard laboratory in K S Rangasamy College of Technology, Department of Biotechnology, Kalvi Nagar, Tiruchengode, Tamil Nadu, India and had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines (Reg. No: 1826 /PO/EReBi/S/15/ CPCSEA).

Induction of Diabetes in Rats: Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of a freshly buffered (0.1 mol/L citrate, pH 4.5) solution of STZ at a dosage of 55 mg/kg body weight. After 72 hours of STZ administration, the tail vein blood was collected to determine fasting blood glucose level with Dr. Morepen GLUCO One glucometer (India). Only rats with hyperglycemia (glucose over 250 mg/dI) were considered diabetic and included in the experiment.

Experimental Design: Rats were divided into six groups, six rats in each group. Group I and II served as normal rats and Diabetic (streptozotocin induced) control rats and were fed with distilled water alone. Group III Diabetic induced rats treated with aqueous extract of seed of *Phaseolus vulgaris* L. (100 mg/kg B.W) 30 days. Group IV Diabetic induced rats treated with aqueous extract of seed of *Phaseolus vulgaris* L. (200 mg/kg B.W) 30 days. Group V: Diabetic induced rats treated with aqueous extract of seed of *Phaseolus vulgaris* L.

Tremendous progress has been executed in the remedy of diabetes by using oral hypoglycemic retailers; however, still, there's a need of look for more modern drugs due to the numerous limitations of the artificial drugs. Phaseolus vulgaris L. (Leguminosae), is a food item of mass consumption in Asian and eastern countries. Numerous parts of the plant were extensively used in Ayurvedic and Unani medication inside the Indian subcontinent for the remedy of diabetes mellitus <sup>7</sup>. In 1995, Roman-Ramos et. a.l, confirmed that the aqueous extract from Phaseolus vulgaris pods possessed antihyperglycemic interest <sup>8</sup>. *Phaseolus vulgaris* L. turned into additionally mentioned to comprise nearly 50 mgs of flavonoids per a hundred g.9 Currently, Venkateswaran S et al. had proved the insulin-stimulatory effect of Phaseolus vulgaris pods from current  $\beta$ -cells in diabetic rats  $^{10}$ . Phaseolus vulgaris L. is the maximum famous bean within the U.S.A and northwestern Mexico 11 and is most often eaten entire (from time to time in broth). or mashed and then refried. Research has indicated pinto beans can decrease the degrees of both HDL and LDL cholesterol. (Pinto beans have also been proven to incorporate the phytoestrogen coumestrol, which has a spread of possible fitness outcomes <sup>12</sup>

The present study is aimed at oxidative pressure and hypoglycemic effect of *Phaseolus vulgaris* L on streptozotocin-diabetic rats. The consequences of *Phaseolus vulgaris* seeds extract is as compared with the hypoglycemic glibenclamide (600 µg/kg body weight) as reference compounds

#### **MATERIALS AND METHODS:**

Collection and Preparation of Plant Material: Plant Material: The healthy seeds of the *Phaseolus vulgaris* L. seed from Erode market, Tamil Nadu, India. The plant specimen was identified by a botanist in department of Agricultural and forestry, Botanical survey, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2018/Tech,3321). The specimens were stored in the Department of Biochemistry, Bharathidasan College of Arts and Science, Erode, Tamil Nadu, India.

**Extraction:** The seeds were cleaned by removing unhealthy seeds, shaded, dried, and then powdered. 50 g of the powder was filled in the thimble and extracted successively using water 500 ml as a

(300mg/kg B.W) 30 days. Group VI Diabetic rats treated with standard drug glibenclamide (600 µg/kgB.W). 15 Extract and the standard drug remedy were given in aqueous solution every day using an intragastric tube for 30 days. Fasting blood glucose was monitored for each week during the experiment.

Sacrifice to Study: The animals were deprived overnight a fast, sacrificed by decapitation and the collected with without blood was and anticoagulant. Tissue samples were instantly dissected out, washed, dried and weighed to measure their antioxidant status.

Body Weight Changes: Rats body weights were evaluated at the start of the experimental duration and after eight weeks using a digital balance. Body weights were measured at the same time during the morning <sup>16</sup>. The experimental animals were observed for signs of abnormalities throughout the period of study.

#### **Biochemical Profiling:**

**Qualitative Determination of Plasma Insulin:** The plasma insulin was assayed by Enzyme Linked Immunosorbent Assay (ELISA) method using Boehringer Mannheim kit <sup>17</sup>. In brief, 0.1 milliliters of plasma was injected into the plastic tubes coated with antibody anti insulin antibodies. Phosphate buffered and anti-insulin POD conjugate was extra to create anti-insulin antibody-POD conjugate. Substrate chemical compound answered was then extra to create indicators reaction. A set of standards were also treated in a similar manner. After the development of coloring the absorbance was read at 420 nm.

**Determination of Serum Lipid Profiles:** Serum triglyceride concentrations of (TG), cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined using commercially available kits supplied by Reactivos GPL, Spain. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula <sup>18</sup>:

$$LDL = [(TC - HDL) - TG/5]$$

**Determination of Serum Liver Functions:** Serum transaminase (AST) and transaminase (ALT) were assayed using kits provided by Biorexfars, UK. Serum alkaline phosphates (ALP) were estimated using kits supplied by Stanbio, USA, whereas serum glutamyl transpeptidase (GGT) and total serum protein (TP) were measured using kits supplied by Reactivos GPL, Spain and Biodiagnostic, Egypt, respectively.

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**Determination of Oxidative Stress Markers for** Hepatic and Pancreatic Tissue: Glutathione peroxidase activity (GSH-Px) was measured according to the method of Necheles et. al., 19 Superoxide dismutase activity (SOD) investigated utilizing the technique of Minami and Yoshikawa <sup>20</sup>. Catalase activity (CAT) was determined by the method of Aebi 21.

Statistical Analysis: The parameter values were analyzed by one-way ANOVA analysis. All the results were expressed as mean  $\pm$  SEM for 6 rats in every group, and P<0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION:** Internationally, diabetes is a complicated biochemical challenge distressing a large number of human individuals. In modern therapeutic alternatives, there are so many aspect results, and secondary failures were found. Still, there's an opening among designing a promising way for the treatment of diabetes; however, through many research, it's been verified already that the plant kingdom is a wealthy source of bioactive compounds with potency towards the therapy for diabetes <sup>22</sup>. we've examined the efficiency of *Phaseolus vulgaris* L by noticing its impacts on the activities of key enzymes and different elements involved in the antioxidant defense systems of manage and induced rats <sup>23</sup>.

In diabetes mellitus, the declined insulin or its performance will result in impaired carbohydrate metabolism. Inside the present study, a reduction in body weight in diabetic rats was discovered which may be the result of deprivation of structural proteins due to unavailability of carbohydrates for usage as a strength supply <sup>24, 25</sup>. A great growth was observed in body weight of diabetic rats treated with Phaseolus vulgaris L extract (2 hundred, three hundred- DC group) as compared to diabetic group which suggests the preventive impact of the extract on degradation of structural proteins.

The diabetic rats have been observed to have higher glucose levels and decrease the level of insulin when in comparison to normal manipulate rats. From the results of the present test, it became observed that treatment *Phaseolus vulgaris* L. extract decreased the serum glucose and multiplied serum insulin in STZ prompted diabetic rats. It's far possible because of stimulation of insulin secretion from remnant pancreatic b-cells, which in flip complements glucose usage by way of peripheral tissues of diabetic rats either by promoting glucose uptake and metabolism or by means of inhibiting hepatic gluconeogenesis <sup>26</sup>.

**Phytochemical Screening for** *Phaseolus vulgaris* **L. Extract:** The preliminary phytochemical screening of *Phaseolus vulgaris* L aqueous extract indicated the presence of carbohydrates, flavonoids, tannins, and phenolic compounds as proven in **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACT OF SEED OF *PHASEOLUS VULGARIS* L.

Phytochemicals	Presence/ absence
Alkaloid	Present
Carbohydrates	present
Flavonoid	Present
Tannin	Absent
Phenol	present

As found in **Fig. 1** and **2**, it's far obvious that diabetes induction causes tremendous growth in blood glucose; lower from GHb and insulin levels due to muscle losing, loss of tissue protein and decreased secretion of the hormone, which is probably due to the destruction of  $\beta$ -cells of pancreas there by way of inhibiting insulin released. Oral administration of aqueous extract of seed of *Phaseolus vulgaris* L. substantially increased the stages of plasma insulin and reduced stages to blood glucose as located. The possible mechanism by which *Phaseolus vulgaris* L. bring

about their hypoglycemic action maybe by using improving the insulin results on plasma by way of growing the secretion of insulin from the prevailing beta cells or with the aid of its release from the certain form.

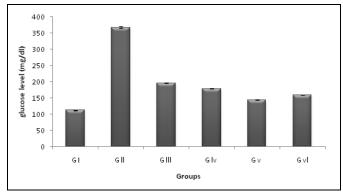


FIG. 1: EFFECT OF DAILY ADMINISTRATION OF AQUEOUS EXTRACT OF SEED OF *PHASEOLUS VULGARIS* L. ON BLOOD SUGAR LEVEL GI- normal control; GII-Diabetic control; GIII-dose 100 mg/kg *P. vulgaris*; GIV-200 mg/kg *P. vulgaris*; GVI-Glibenclamide

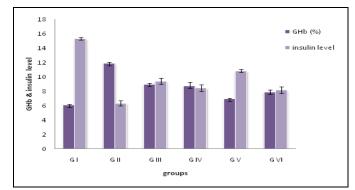


FIG. 2: EFFECT OF AQUEOUS EXTRACT OF SEED OF *PHASEOLUS VULGARIS* L. IN GHB AND INSULIN LEVEL GI- normal manage; GII-Diabetic control; GIII-dose 100 mg/kg *P. vulgaris*; GIV-200 mg/kg *P. vulgaris*; GV-300 mg/kg *P. vulgaris*; GVI- Glibenclamide

**Table 2** suggests the levels of serum lipid profile of rats in exclusive experimental groups. Rats in DC group displayed a considerable growth within the levels of TG, TC, and LDL-C in contrast with the NC group.

TABLE 2: EFFECT AQUEOUS EXTRACT OF SEED OF PHASEOLUS VULGARIS L. ON LIPID PROFILE

Groups	TG	TC	LDL	VLDL	HDL
control	$85.23 \pm 0.55^{a}$	$80.48 \pm 0.85^{a}$	$32.51 \pm 0.34^{a}$	$19.05 \pm 0.26^{a}$	$29.84 \pm 0.39^{a}$
Diabetic(DC)	$180.72 \pm 0.28^{b}$	$179.18 \pm 1.22^{b}$	$169.07 \pm 2.17^{b}$	$41.32 \pm 0.38b$	$15.36 \pm 0.07^{b}$
100 mg/kg	$158.4 \pm 3.83^{ab}$	$167.13 \pm 2.33^{ab}$	$160.17 \pm 5.08^{ab}$	$30.19 \pm 0.29^{ab}$	$20.49 \pm 0.35^{ab}$
200 mg/kg	$152.32 \pm 10.29^{ab}$	$160.42 \pm 8.83^{ab}$	$151.07 \pm 15.06^{ab}$	$32.1 \pm 1.40^{ab}$	$21.11 \pm 1.47^{ab}$
300 mg/kg	$90.9 \pm 4.01^{ab}$	$100.08 \pm 1.66^{ab}$	$59.33 \pm 5.41^{ab}$	$21.55 \pm 0.49^{ab}$	$26.6 \pm 0.33^{ab}$
Standard	$122.08 \pm 5.53^{ab}$	$127.87 \pm 2.76^{ab}$	$127.53 \pm 20.68^{ab}$	$27.37 \pm 0.45^{ab}$	$24.69 \pm 0.36^{ab}$

Data are expressed as mean  $\pm$  SEM (n = 6). Values with different superscripts down the column are significantly different at P < 0.05. a Statistically different from NC group. b Statistically different from DC group.

But, serum HDL-C level of rats in the DC group was significantly decrease than that of rats in the NC group. remedy with *Phaseolus vulgaris* L extract in 200-DC and 300-DC groups confirmed a substantial lower inside the levels of serum TG, TC, and LDL-C and simultaneous significant growth inside the level of HDL-C when in comparison with the DC group. Most effective the serum HDL-C return to the basal level of the NC group, the serum TG, LDL-C and TC did no longer return to the basal level of NC group. In diabetes, hyperglycemia is observed with dyslipidemia representing a threat issue for coronary heart illnesses.

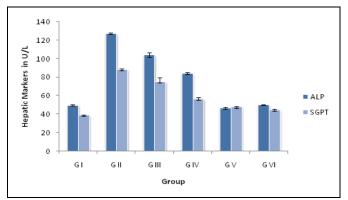


FIG. 3: IMPACT OF AQUEOUS EXTRACT OF SEED OF *PHASEOLUS VULGARIS* L. IN HEPATIC MARKER GI- normal manipulate; GII-Diabetic manipulate; GIII-dose one hundred mg/kg *P. vulgaris*; GIV-two hundred mg/kg *P. vulgaris*; GV-three hundred mg/kg *P. vulgaris*; GVI-Glibenclamide

The strange excessive level of blood serum lipids is mainly because of the unrestrained moves of lipolytic hormones on the fat depots, chiefly because of the action of hypoglycemic agent. underneath regular occasions, insulin turns on the enzyme lipoprotein lipase, which hydrolyzes TGs. However, in a diabetic state, conjugated protein enzyme isn't always activated because of hypoglycemic agent deficiency, resulting hypertriglyceridemia, and insulin deficiency is

likewise associated with hypercholesterolemia due to metabolic abnormalities <sup>27</sup>.

TGs stimulate the secretion of very-low-density lipoprotein LDL cholesterol, and such an increase in very-low-density lipoprotein LDL cholesterol debris reduces the HDL-C stage and will increase the LDL-C particles <sup>28</sup>. The function features of diabetic dyslipidemia are increased from serum TG, TC, LDL-C, and fall in HDL-C level <sup>29</sup>. In our look at, the altered serum lipid profile become determined in diabetic rats. This finding is in the correlation between the findings of Pepato et al., and Sharma et al., 30, 31. This altered serum lipid profile became reversed closer to normal after the management of *Phaseolus vulgaris* L extracts with both doses. Hence, the extract may be helpful in enhancing lipid metabolism so that you can in turn, help to prevent diabetic complications consisting of coronary coronary heart illnesses and atherosclerosis.

It's been properly established that extended levels to SGPT and ALP are indicative of cellular leakage and lack of purposeful integrity of the hepatic cellular membranes implying hepatocellular harm <sup>32</sup> within the present observe, the injection of STZ induces hepatocellular harm, that's one of the function changes in diabetes as evidenced by high serum ranges of SGPT and ALP in diabetic group compared to the everyday manipulate, suggesting possible damage to the liver. Liver broken in diabetic rats changed into confirmed but diabetic corporations handled with *Phaseolus vulgaris* L extract from 300-DC, 200-DC, and 100-DC groups showed a significant reduction in the levels of those enzymes when compared to the diabetic untreated control, which therefore alleviated the damage because of STZ as confirmed by way of hepatocytes morphology.

TABLE 3: OUTCOMES ON ENZYMATIC ANTIOXIDANTS OF SOD, CAT AND GPX IN BLOOD

Groups	SOD 50% inhibition of epinephrine auto	CAT µmol H2O2/min/	GPx μmol glutathione/min/ mg	
	oxidation/ min	mg protein	protein	
control	$15.24 \pm 0.18^{a}$	$38.51\pm0.04^{a}$	$6.35 \pm 0.06^{a}$	
Diabetic(DC)	$31.40 \pm 0.32^{b}$	51.11±0.31 <sup>b</sup>	$14.41 \pm 0.05^{b}$	
100 mg/kg	$26.73 \pm 0.75^{\circ}$	$49.54\pm0.83^{\circ}$	$11.47 \pm 0.46^{c}$	
200 mg/kg	$23.43 \pm 0.46^{\circ}$	$45.25\pm0.36^{\circ}$	$9.58 \pm 0.36^{d}$	
300 mg/kg	$19.13 \pm 0.21^{d}$	$42.30\pm0.36^{c}$	$8.09 \pm 0.26^{\rm e}$	
Standard	$17.93 \pm 0.28^{e}$	$40.24\pm0.42^{c}$	$7.48 \pm 0.08^{\rm f}$	

Statistics are expressed as imply  $\pm$  SEM (n = 6). a, b, c, d, e, f Values with exclusive superscripts in the column are extensively one-of-a-kind at P < 0.05

Because of this *Phaseolus vulgaris* L has some hepatoprotective potentials for diabetic rats via reducing serum SGPT and ALP stages. Under the circumstance of intense aerophilous strain,

unfastened radical technology leads to protein amendment. Proteins can be damaged directly by way of specific interactions of free radicals with unique prone amino acids <sup>33</sup>.

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TABLE 4: EFFECT OF AQUEOUS EXTRACT OF SEED OF *PHASEOLUS VULGARIS* L. ON OXIDATIVE STRESS MARKERS ON HEPATIC AND PANCREATIC TISSUE

SOD (unit/mg protein)		CAT (unit/mg protein)		
Groups	Pancreases	liver	pancreases	liver
Control	$0.60 \pm 0.01^{a}$	$0.44\pm0.01^{a}$	$0.59 \pm 0.01^{a}$	$0.62 \pm 0.01^{a}$
Diabetic(DC)	$2.13\pm0.04^{b}$	$2.15\pm0.01^{b}$	$3.26 \pm 0.02^{b}$	$3.04 \pm 0.01^{b}$
100 mg/kg P. vulgaris	$1.32\pm0.04^{c}$	$1.10 \pm 0.12^{c}$	$2.19 \pm 0.20^{c}$	$1.45\pm0.07^{c}$
200 mg/kg P. vulgaris	$1.15\pm0.14^{c}$	$1.37 \pm 0.20^{c}$	$1.89 \pm 0.16^{d}$	$1.36 \pm 0.17^{c}$
300 mg/kg P. vulgaris	$0.61\pm0.02^{a}$	$0.64 \pm 0.01^{d}$	$1.35 \pm 0.03^{d}$	$0.87 \pm 0.03^{d}$
standard	$0.93 \pm 0.13^{d}$	$0.97 \pm 0.06^{\rm e}$	$1.7 \pm 0.17^{d}$	0.92±0.10 <sup>e</sup>

Data are expressed as mean  $\pm$  SEM (n = 6). abcd Values with different superscripts within the column are significantly different at P < 0.05

Oxidative stress is normally encouraged as mechanism underlying polygenic ailment and diabetic complications, which ends from an imbalance among radical producing and radical scavenging structures <sup>34</sup>. Antioxidant enzymes in addition to non-enzymatic antioxidants are first line of protection in opposition to ROS prompted oxidative harm to a dwelling organism <sup>35</sup>. SOD, CAT and GSH-Px are the three predominant scavenging enzymes that eliminate the poisonous unfastened radicals *in-vivo* <sup>36</sup>. SOD protects tissues against oxygen unfastened radicals by means of catalyzing the removal of superoxide radicals, changing it into H<sub>2</sub>O<sub>2</sub> and molecular oxygen, which each damage the mobile membrane and other organic structures. CAT is a haem protein that's chargeable for the detoxification of giant quantities of H<sub>2</sub>O<sub>2</sub> <sup>37</sup>. GSH-Px plays a primary position inside the catabolism of H<sub>2</sub>O<sub>2</sub> and the cleansing of endogenous metabolic peroxides and hydroperoxides, which catalyzes GSH. Glutathione features as a free radical scavenger and is an important co-substrate for GSH-Px <sup>39</sup>.

The reduced interest of antioxidant (GSH-Px, SOD and CAT) enzymes became determined in the liver and pancreatic tissues of diabetic rats. Those consequences are in agreement with Cheng *et al.*, <sup>40</sup> It turned into recommended that decreased antioxidant enzyme interest in the DC group can be because of glucan of these enzymes, which happened at constantly extended blood glucose stages <sup>41</sup>. However, administration of *Phaseolus vulgaris* L extract from 300-DC, 200-DC, and 100-DC groups multiplied the GSH-Px, SOD, and CAT

activities in the liver and pancreas of diabetic rats. Robertson *et al.*, demonstrates that antioxidants were shown to break the worsening of diabetes via improving b-cells characteristic in animal fashions and cautioned that improving antioxidant defense mechanisms in pancreatic islets may be a treasured pharmacologic method to handling diabetes <sup>42</sup>.

CONCLUSION: In consideration of the results we obtained in study observations, it was clearly indicated that *Phaseolus vulgaris* L. has the tendency to prevent the liver and renal tissues from the damage caused by the oxidative stress during diabetes in STZ induced experimental rats. It has a good impact on the enzymatic (SOD, CAT, and GPx) activity. On the whole, experimental evidence obtained from this study is encouraging enough to warrant further studies on the seed extract of this plant to find out its mechanism of action and to establish its therapeutic potential in the prophylaxis and/or treatment of diabetes and diabetic complications.

#### **ACKNOWLEDGMENT: Nil**

#### CONFLICTS OF INTEREST STATEMENT:

We declare that we've no conflict of interest

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