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INVESTIGATION OF HYPERGLYCAEMIC ACTIVITY OF POLYHERBAL FORMULATION ON BLOOD SERUM GLUCOSE LEVEL IN ALLOXAN INDUCED DIABETIC RATS

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Abstract:
The blood glucose lowering activity of the Polyherbal preparation-I (1:1:1 of Wheat germ oil, Coraidrumsativum and Aloe vera) was studied in normal rats after oral administration at doses of 1.0 ml/kg and 2.0 ml/kg and Polyherbal preparation-I,II (Wheat germ oil, fresh juice of Coriandrumsativum and Aloe vera in the ratio of 1:2:1), and III (Wheat germ oil, fresh juice of Coriandrumsativum and Aloe vera in the ratio of 2:2:1) onalloxan-induced diabetic rats, after oral administration at doses of 1.0 ml/kg and 2.0 ml/kg. Blood samples were collected from tail vein method at 0, 0.5, 1, 2, 4, 8, 12, and 24 h in normal rats and in diabetic rats at 0, 1, 3, 7, 15 and 30 days. Blood plasma glucose was estimated by GOD/POD (Glucose oxidase and peroxidase) method. The data was compared statistically by using one way ANOVA method followed by Dunnett multiple component test. Statistical significance was set at P<0.05.
***KEY WORDS**: Blood plasma glucose level, Alloxan-induced diabetes.*

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INTRODUCTION:

Diabetes is a condition where the body fails to utilize the ingested glucose properly. This could be due to lack of the hormone insulin or because the insulin that is available is not working effectively. Every time we eat sugary or starchy food, the amount of glucose available to the body rockets. Yet the levels of glucose in the bloodstream are maintained within narrow limits by two key hormones – insulin and glucagon – working to prevent hyperglycemia (abnormally high glucose levels) or hypoglycemia (low glucose)[1,2].Insulin helps the sugar to leave the blood and go into our body cells, where it is used as a kind of fuel. When this happens the way it should, the level of sugar in the blood goes down and our bodies have the energy for a full and active life. In people with diabetes, this system does not work. When you have diabetes, your body cannot make energy from the food you eat. Sugar stays in the blood instead of going into the cells of the body. Diabetes is a lifelong serious disease and should be treated as such. Left unchecked, it shortens life. It is not a condition that goes away[3].

Diabetes is defined as a state in which homeostasis of carbohydrate, lipid and protein metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis dose not returns to normal and continues for a protracted period of time, it leads to hyperglycaemia that in due course turns into a syndrome called diabetes mellitus[4].

MATERIALS AND METHODS

5.1 Material selection:

Collection of plant materials:

Aloe vera leaves were washed properly to remove the dirt. After washing remove the skin of leaves, gel were separated ant collected. Fresh juice of *Coriandrum sativum* obtained by crushing leaves in mortar and squeezed the crushed material by means of fine cloth to separate the juice and collected the fresh juice of leaves.

Chemicals used:

List of chemicals used:

	Supplied by:
Alloxan	s.d. fine-chem limited, Mumbai
Sodium chloride (Nacl)	s.d. fine-chem limited, Mumbai
Glibenclamide Tablet (5mg)	Nicholas Piramal india limited.
Acacia	s.d. fine-chem limited, Mumbai
Diagnostic Kit:	*Supplied by:*
Glucose Estimation Kit	Qualigens diagnostics

Preparation of solutions and Test samples:

Preparation of Alloxan monohydrate 5% Solution: Alloxan monohydrate, 250mg was dissolved in 5.0 ml of 0.9% sodium chloride to give 5.0% of Alloxan solution and injected immediately through i.p route within five minutes to avoid degradation.

Glibenclamide Solution*:*Glibenclamide tablet of 5 mg was dissolved in 83.33 ml of distilled water to give 60 µg/ml solution. This solution was administered at a dose[200]of 600 µg/ kg body weight using clean and dry oral feeding needle for 30 days.

Preparation of Polyherbal preparation[201] -I, II and III:

Polyherbal preparation-I: It consists of *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* in the ratio of 1:1:1. Formulation was prepared by

using acacia [202,203,204]as a binding agent.

Take 2.0 ml of *wheat germ oil*. Transfer it into a dry mortar. Add 500 mg of gum acacia powder, mixed thoroughly by trituration. Add 1.0 ml of *Coriander* juice at the time of continuous trituration until a light green product with clicking sound is produced. Primary emulsion was formed. Add *Aloe vera* gel 2.0 ml with remaining 1.0 ml of coriander juice separately. Add this solution at a time to primary emulsion with continuous and rapid trituration. The emulsion was used for study.

Polyherbal preparation-II: It consists of *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* in the ratio of 1:2:1. Formulation was prepared by using acacia as a binding agent.

Take 1.0 ml of *wheat germ oil*. Transfer it into a dry mortar. Add 500 mg of gum acacia powder, mixed thoroughly by trituration. Add 1.0 ml of *Coriander* juice at the time of continuous trituration until a light green product with clicking sound is produced. Primary emulsion was formed. Add *Aloe vera* gel 1.0 ml with remaining 1.0 ml of coriander juice separately. Add this solution at a time to primary emulsion with continuous and rapid trituration. The emulsion was used for study.

Polyherbal preparation-III: It consists of *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* in the ratio of 2:2:1. Formulation was prepared by using acacia as a binding agent.

Take 2.0 ml of *wheat germ oil*. Transfer it into a dry mortar. Add 250 mg of gum acacia powder, mixed thoroughly by trituration. Add 1.0 ml of *Coriander* juice at the time of continuous trituration until a light green product with clicking sound is produced. Primary emulsion was formed. Add *Aloe vera* gel 1.0 ml with remaining 1.0 ml of coriander juice separately. Add this solution at a time to primary emulsion with continuous and rapid trituration. The emulsion was used for study.

Experimental Animals:

Animals

For pharmacological experiments, Wistar albino rats (160-250g) of either sex were used.

Housing of Animal

Animals were maintained in suitable nutritional and environmental condition throughout the experiment. They were provided with food, water ad libitum. The bedding material of the cages was changed every day. The animals were maintained under natural day and night cycle.

5.2 Experimental Method:

Experimentally induced diabetes mellitus[205,206,207,208,209]:

Wistar rats (160–250 g) were fasted for 14 h before challenging with single injection (i.p) of Alloxan monohydrate 5.0%[210,211], freshly prepared and injected within 5 min of preparation to prevent degradation at a dose of 150 mg/kg, i.p[212,213,214,215]. After administration of alloxan the animals had free access to feed and water *ad libidum*. The blood glucose was measured 72 h of alloxanisation. After one week when the condition of diabetes was stabilized, the animals were fasted again for 14 h before blood collection withdrawal from retro orbital plexus. The rats with fasting serum glucose level of above 200 mg/dl were considered diabetic and included in the study.

Experimental design:

The experimental setup consist of twelve groups, each group consist of five animals.

Group-I: Vehicle control received saline solution (0.9%w/v Nacl).

Group-II: Normal rats received Glibenclamide as standard, single dose.

(Dose: 600 µg/kg).

Group-III: Normal rats received Polyherbal preparation-I, single dose.

(Dose: 1.0 ml/kg)

Group-IV: Normal rats received Polyherbal preparation-I, single dose.

(Dose: 2.0 ml/kg).

Group-V: Alloxan induced rats received saline solution (0.9%w/v Nacl).

Group-VI: Diabetic rats given Glibenclamide as standard, orally once daily.

(Dose: 600 µg/kg).

Group-VII: Diabetic rats given Polyherbal preparation-I, orally once daily.

(Dose: 1.0 ml/kg).

Group-VIII: Diabetic rats given Polyherbal preparation-I, orally once daily.

(Dose: 2.0 ml/kg).

Group-IX: Diabetic rats given Polyherbal preparation-II, orally once daily.

(Dose: 1.0 ml/kg).

Group-X: Diabetic rats given Polyherbal preparation-II, orally once daily.

(Dose: 2.0 ml/kg).

Group-XI: Diabetic rats given Polyherbal preparation-III, orally once daily.

(Dose: 1.0 ml/kg).

Group-XII: Diabetic rats given Polyherbal preparation-II, orally once daily.

(Dose: 2.0 ml/kg).

Care of Diabetic Animals:

Since diabetic animals drink large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day, and, in some circumstances, more than once per day. Diabetic rats should have sufficient food and water; therefore only three diabetic rats have been housed per cage to avoid competition for feed and water.

Collection of blood plasma samples:

The rat was placed on the working table, a mortar was inverted on it, and no gap was maintained in between mortar and the edge of the working platform. The tail was pulled out from the mortar's beak gap and the tail was depilated. The tail was cleaned with spirit and allowed to dry; tail vein was dilated by focusing a low voltage electric lamp at the tip of tail for few seconds. After the dilation of the vein the blood was drawn by tail vein method from rats (fasted for 14 h) on different occasion, i.e., at 0, 0.5, 1, 2, 4, 8, 12, and 24 h after oral administration of drug in groups I to IV, Where as in group V to XII at 0, 1, 3, 7, 15 and 30 days. The blood samples were collected in centrifugation tube and add heparin (0.2 ml for 1.0 ml blood) as an anticoagulant. The plasma was obtained by centrifuging the blood samples at 3000 rpm for 10 mins, decanting supernatant fluid into the clean, dry test tube. 10µl of the plasma was collected and the blood glucose level was estimated by GOD/POD method.

5.3 Parameter analyzed:

Fasting Blood Plasma Glucose Estimation:

Blood plasma glucose was estimated by the GOD/POD (Glucose oxidase and peroxidase) method[216] using the GLUCOSE–GOD/POD kit (GSK Qualisystems; AR106).

GOD /POD method

The older methods were based on the reducing property of glucose. But these methods do not measure the true glucose because of interferences. Subsequently other chemical and enzymatic methods were developed to overcome this problem. The GOD/POD method is one such evolved method by Trinder in 1964. This method is precise, simple, and single stepped, rapid, safe and reliable. Hence, in the present study, we have adopted this method. Trinder's method (1964) utilizes two enzymes Glucose oxidase (GOD) and peroxidase (POD) along with the chromogen 4- amino antipyrine and phenol. This method is intended for invitro quantitative determination of glucose in serum/plasma and CSF. There was no interference due to the substances like creatinine, fructose, galactose, reduced glutathione, ascorbic acid and xylose. Hemoglobin or bilirubin up to 10mg% does not affect the test.

Principle

Glucose is oxidized by the enzyme Glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Glucose present in the blood is oxidized by the enzyme Glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide further reacts with 4-aminoantipyrine and phenol in presence of the enzyme peroxidase (POD) to undergo oxidation to produce a red colour quinoneimine dye.

The intensity of the colour produced is directly proportional to glucose concentration in the sample.

GOD

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{} \text{D-Gluconic acid} + H_2O_2$$

POD

$$H_2O_2 + \text{4-aminoantipyrinephenol} \xrightarrow{} \text{Quinoneiminedye} + H_2O$$

Reagents used in the glucose kit

1. Enzyme reagent powder	-	5vials
2. Buffer solution	-	5x100ml
3. Standard Glucose (100mg/dl)	-	1Vial (3ml)

Preparation of working reagent

1 vial of enzyme powder was transferred to 1 bottle (100ml) of buffer solution and mixed gently to dissolve which is ready to use.

Reagent storage and stability

- All the reagents were stored at 2-8°C, which is stable till the expiry date, reagents were not freeze.
- Capped reconstituted reagents are stable for 30 days at 2-8°C.
- Reconstituted enzyme reagents when stored at 2-8°C develops a slight pink colour, however this does not affect the performance of the enzyme reagent.
- The reagents should not be used if caking is observed due to possible moisture penetration.

Procedure

Pipette out the solutions into clean, dry test tubes and labelled as blank (B), Standard (S), and test (T).Distilled water was considered as blank solution. To 1.0 ml of the reagent, 10 µl of standard glucose (100 mg/ dl) was added and incubated for 15 min at 370º C. This incubated mixture was aspirated and concentration of standard was calibrated to show a value of 100 mg/ dl. This process is duplicated to confirm the calibration value and was considered as correct if the value was within 5 % of the original value.

The fasting blood plasma glucose was estimated by adding 10 µl of the plasma sample to 1.0 ml of the reagent, mixed well and incubated at 37^0 C for 15 min. This incubated mixture was aspirated and absorbance recorded against a reagent blank at 510 nm using Photometer (GSK Qualisystems; AR106).

TABLE 1: General Procedure of GOD/POD for the estimation of glucose in plasma

Sl. No	Content	Blank	Standard	Test
1	Working reagent enzyme	1.0 ml	1.0 ml	1.0 ml
2	Distilled water	10 µl	10 µl	10 µl
3	Standard glucose	-	10 µl	-
4	Serum (sample)	-	-	10 µl

6. RESULT:

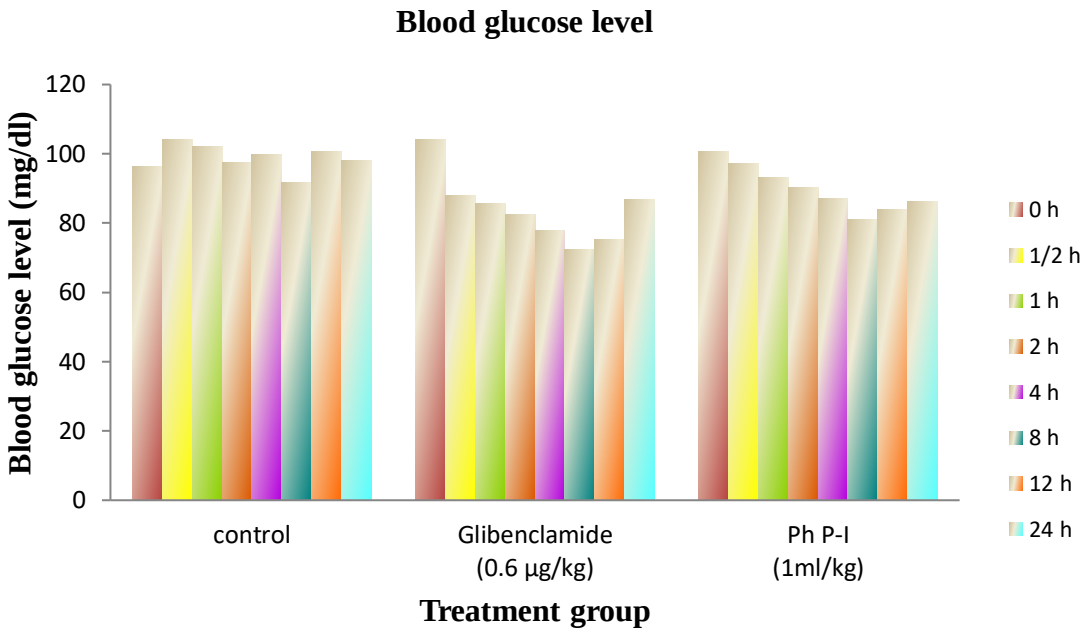
Fasting Blood Plasma Glucose:

The fasting blood plasma glucose of the different groups of normal animals during the period of study is given in table No 6 and presented in graph 2, which shows that the mean (± SEM) fasting plasma glucose values of the normal control group of rats was 96.4±1.364 mg/dl,104.2±1.497 mg/dl, 102±1.342 mg/dl, 97.4±1.470 mg/dl, 99.8±1.625 mg/dl, 91.6±1.503 mg/dl,100.6±0.95 mg/dl and98.0±1.125 mg/dl on day 0, ½, 1, 2, 4, 8, 12 and 24h respectively. The above values show that the fasting plasma glucose in the normal group of rats was maintained within the normal range throughout the period of study.

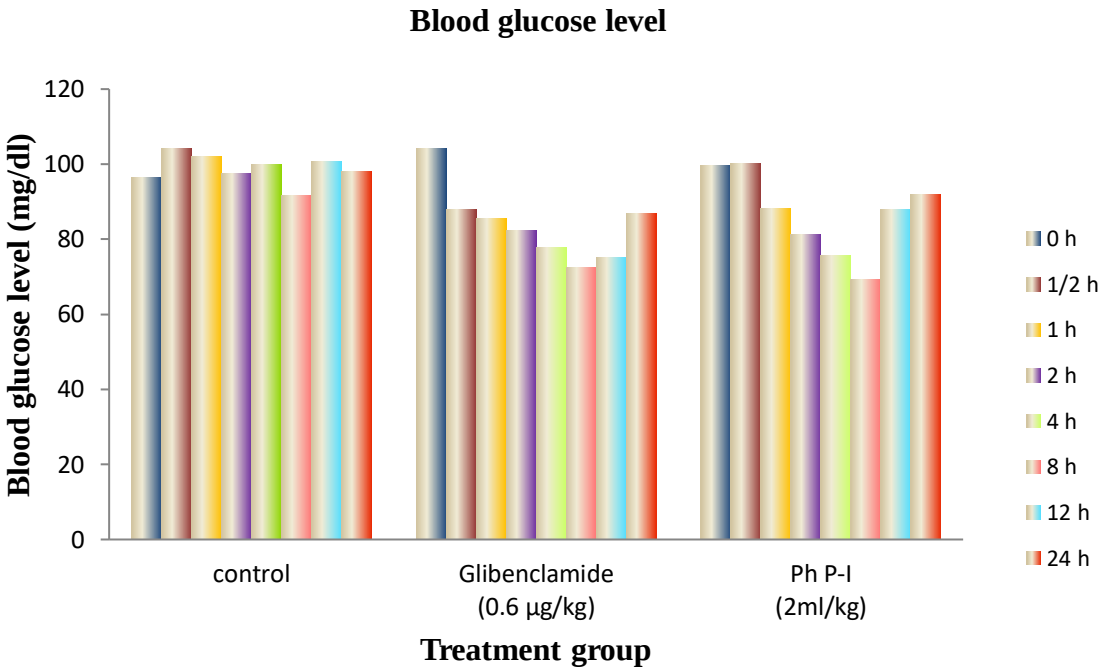
Groups	Treatment	Dose	Fasting Blood Plasma Glucose (mg/dl) Mean ± S.E.M							
			0 h	½ h	1 h	2h	4h	8h	12h	24 h
Group-I	Control (0.9% saline)	2.0 ml/kg	96.4± 1.364	104.2± 1.497	102.0± 1.342	97.4± 1.470	99.8±1.62 5	91.6± 1.503	100.6±0 .95	98.0±1. 125

Group- II	Glibenclamide	0.6 µg/kg	104.2±2.835[a]	88.0±2.302[b]	85.6±2.713[b]	82.4±2.441[b]	77.8±2.782[b]	72.4±2.542[b]	75.2±2.212[b]	86.8±3.554[b]
Group-III	Ph p-I	1.0 ml/kg	100.8±1.114	97.2±1.319[a]	93.2±1.114[a]	90.4±1.166[a]	87.0±1.265[b]	81.0±1.342[b]	83.8±1.108[b]	86.3±1.687[b]
Group-IV	Ph p-I	2.0 ml/kg	99.6±1.364	100.2±0.860	88.2±1.562b	81.4±1.860b	75.6±2.561[b]	69.4±2.441[b]	88.0±1.581[b]	91.8±0.734
One-way ANOVA		F	3.195	18.959	16.261	17.414	26.122	24.097	49.15	6.542
		df	3, 16	3, 16	3, 16	3, 16	3, 16	3, 16	3, 16	3, 19
		P	0.0519	<0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0032

Graph 2:Effect of Polyherbal preparation-I (1 ml/kg) on fasting blood plasma glucose levels after oral administration in normal rats.



Graph 3:Effect of Polyherbal preparation-I (2 ml/kg) on fasting blood plasma glucose levels after oral administration in normal rats.



CONCLUSION:

The present study on the Polyherbal preparation-I, II and III formulated from three different plants i.e. *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* gel in three different ratio's were started with an expectation and objective to explore the possibility of the drug to exert antidiabetic effect. In this study, alloxan induced diabetic animals were used. Antidiabetic animals were treated with different doses of Polyherbal preparation-I, II and III to assess the effect of the drugs. After treatment, to analyze the effect of the drug glucose estimation test was performed. The data obtained were satisfactory and conclusive so as and to accomplish our objectives. In conclusion, the present data indicated that the Polyherbal preparation-I i.e. *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* in the ratio of 1:1:1 showed significant glucose lowering effect in normal rats and Polyherbal preparation-I, II i.e. *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* in the ratio of 1:2:1 and Polyherbal preparation-III i.e. *Wheat germ oil*, fresh juice of *Coriandrum sativum* and *Aloe vera* in the ratio of 2:2:1 showed significant glucose lowering effect in alloxan induced diabetic rats.

This study suggests that Polyherbal preparation-I, II, and III posses antidiabetic activity and is going to be a promising antidiabetic preparation for masses. Exact mechanism underlying this effect is not clear, but apparently may be due to preserving β-cell function in diabetic rat. Further studies are needed to elucidate the mechanism of action and to know the active principle/s involved in producing the effect.

Keeping in view the tremendous pharmacological activities these Polyherbal preparation possess, it may be utilized to alleviate the symptoms of diabetes,

however it has to be confirmed by clinical trials before put into the therapy.

REFERENCE:

1. King H, Aubert RE, Herman WH. Global burden of diabetes 1995-2025. *Diabetes Care.* 1998;21:1414–31. [PubMed] [Google Scholar]

2. Kameswara Rao B, Giri R, Kesavalu MM, Rao A. Herbal medicine in the management of diabetes mellitus. *Manphar Vaidhya Patrika.* 1997;1:33–5. [Google Scholar]

3. Cryer PE. Hypoglycemic agents. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, editors. *Harrison's principles of internal medicine.* 15th ed. Vol 2. McGraw-Hill; 2001. pp. 2138–43. [Google Scholar]

4. Davis SN, Granner DK. Insulin, oral hypoglycemic agents and endocrine pancreas. In: Gilman AG, Goodman LS, Rall TW, Murad F, editors. *The pharmacological basis of therapeutics.* 10th ed. New York: McGraw-Hill; 2001. pp. 1679–714. [Google Scholar]

5. Winocour PH, Ishola M, Durrington PN. Validation of the friedewald's formula for the measurement of low density lipoprotein cholesterol in insulin dependant diabetes mellitus. *Clin Chim Acta.* 1989;179:79–83. [PubMed] [Google Scholar]

6. Trinder P. Determination of blood glucose using an oxidase peroxidase system with a non carcinogen chromogen. *J Clin Pathol.* 1969;22:158. [PMC free article] [PubMed] [Google Scholar]

7. Siedel J, Hagele EO, Ziegenhorn J, Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem.* 1983;29:1075–80. [PubMed] [Google Scholar]

8. Fossati P. Enzymatic determination of serum triglycerides. *Principle Clin Chem.* 1982;28:2077. [PubMed] [Google Scholar]

9. Gidez WM, Webb M. Revision of cholesterol determination. *J Biochem.* 1950;187:97–106. [PubMed] [Google Scholar]

10. Palton CJ, Crouch SR. spectrophotometric and kinetics investigation of the berthelot reaction for the determination of ammonia. *Anal Chem.* 1977;49:464–9. [Google Scholar]

11. Bartels A, Boehmer M. Microdetermination of creatinine. *Clin Chim Acta.* 1971;32:81–5. [PubMed] [Google Scholar]

12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–8. [PubMed] [Google Scholar]

13. Beutler E, Kelley BM. The effect of sodium nirate on RBC glutathione. *Experientia.* 1963;19:96–7. [PubMed] [Google Scholar]

14. Mishra SS, Sharma AL, Sharma S. Phytochemical and pharmacological studies on Launaea nudicaulis. *Indian J Pharmacol.* 1979;11:63. [Google Scholar]

15. Aeibi H. In: *Catalase: Methods of enzymatic analysis.* Bergmeyer HV, editor. Chemie, Weinheim, Germany: 1974. pp. 673–84. [Google Scholar]

16. Rodrigues B, McNeill JH. Cardiac function in spontaneously hypertensive diabetic rats. *Am J Physiol.* 1986;251:H571–80. [PubMed] [Google Scholar]

17. Hofteizer B, Carpenter AM. Comparison of STZ

induced diabetes in rats, including volumetric quantization of pancreatic islet cells. *Diabetologia.* 1973;9:178–84. [PubMed] [Google Scholar]

18. Murali B, Goyal RK. Effect of chronic treatment with losartan on streptozotocin induced diabetic rats. *Indian J Exp Biol*. 2002;40:31–4. [PubMed] [Google Scholar]

19. Babu V, Gangadevi T, Subramoniam A. Antidiabetic activity of ethanol extract of Cassia Kleinii leaf in streptozotocin induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J Pharmacol*. 2003;35:290–6. [Google Scholar]