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RESEARCH ARTICLE

ANTI-HYPERGLYCAEMIC ACTIVITY OF A POLYHERBAL FORMULATION AND ITS PROTECTIVE ROLE AGAINST HEPATOPATHY IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present investigation was undertaken to evaluate antihyperglycaemic activity of an aqueous extract of *Diabcap* (a polyherbal formulation) and to ascertain its role in hepatoprotection in Streptozotocin (STZ) (60mg/kg, ip single dose) induced diabetes in rats as though potentially uncommon yet diabetic hepatopathy may be an apt organ to be targeted in diabetes. The rats were randomly divided in diabetic and non-diabetic groups. *Diabcap* was administered in two different doses and results were compared with metformin. The diabetic rats showed a marked elevation in fasting blood glucose level, aspartate transaminase, alanine transaminase, serum bilirubin, serum alkaline phosphatase, serum cholesterol & triglyceride. Administration of *Diabcap* significantly ($p<0.001$) reduced the blood glucose level in 300mg/Kg dose of hyperglycaemic rats and showed good results in improving liver function test and reduces the triglyceride and cholesterol level of STZ induced diabetic rats (SIDRs). Histological examination of the liver of normal, diabetic control and drug treated rats were in the same direction with biochemical findings. *In vivo* toxicity was also performed in albino rats. In the acute oral toxicity each group of rats was orally given a single dose of 2gm/Kg and in sub-acute toxicity rats were administered with 5, 50, 300mg/Kg body weight (BW) of *Diabcap* once daily for 28 days. This study provides compelling evidence for a holistic approach of *Diabcap* in amelioration of diabetes linked manifestation or dysregulations. Finally it was concluded that besides acting as an effective antihyperglycaemic agent it can also be used against diabetic hepatopathy.

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia (Longo *et al*., 2011) characterized by; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease (Davis, 2006). Development of modern medicine has provided various groups of pharmacotherapeutic agent but they have their own limitations, besides their high cost severe adverse reactions and toxicity can be caused due to the prolonged use of many of these drugs and therefore use of herbal medicines have been recommended for treatment of diabetes (Pandit *et al*., 2010). It is well recognized now that prolonged hyperglycemia can cause many complications like retinopathy, neuropathy and nephropathy, yet there is recondite information in terms of researches in diabetic hepatopathy (Porepa *et al*., 2010). According to the recent researches almost in every diabetic patient an array of liver diseases can be easily found, in fact diabetes mellitus hasten the occurrence of liver disorders by altering its physiology (Banerjee *et al*., 2008, Tziomalos *et al*., 2012, Cusi, 2009, Arase *et al*., 2013). Thus making liver a suitable organ for which the preventive measures should be taken, akin to that taken for other diabetic complications. Studies have shown that synthetic drugs particularly insulin, insulin secretagogues and thiazolidinedione used in diabetes mellitus can cause hepatotoxicity in long term (Kesavanarayanan *et al*., 2013). Over the past few years the use of herbal medicines have become increasingly popular as they hold potential in curing diabetes and liver toxicity alone or as an adjuvant with oral hypoglycaemic agents or even with insulin. Several medicinal plants in the form of compound drugs have been used from ages (Yadav *et al*., 2012). Moreover the investigations on therapeutic benefits of medicinal plants on diabetes have become more important after the recommendations made by WHO (Gupta *et al*., 2005). *Berberis aristata* (commonly called as *daruharidra*) has anti-hyperglycaemic property and its formulations are useful in various diseases, one of which is diabetes mellitus (Potdar *et al*., 2012) and it is used as a tonic remedy for liver and heart for its antihepatotoxic activity (Janbaz and Gilani, 2000). Root extract of *Berberis aristata* regulates the glucose homeostasis through decreased gluconeogenesis and oxidative

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stress (Singh and Kakkar, 2009). *Cyperus rotundus* (commonly *Nagarmotha*) has many ethno botanical uses and in traditional ayurvedic system and it has been used in diabetes mellitus (Raut and Gaikwad, 2006). *Cedrus deodara* (commonly *devdaru*) has been advocated in *Ayurveda* lexicon in tackling diabetes and it is reported to have wide range of activity such as antihyperlipidemic, immunomodulatory, anti-inflammatory (Patil *et al*., 2011). In India *Emblica officinalis* (commonly *amla*) has been extensively used against diabetes and inflammation (Nain *et al*., 2012, Sabu and Kuttan, 2002) Fruit juice of *Emblica* is also reported to exhibit hypolipidaemic activity in cholesterol fed rabbits (Mathur *et al*., 1996). Many reports also suggests that it is effective in inhibiting the liver toxicity (Achliya *et al*., 2004, Jose and Kuttan, 2000). *Terminalia chebula* (commonly *Harad*) has been reported to exhibit a variety of biological activities, including antidiabetic, renoprotective and antihyperlipidemic (Rao and Nammi, 2006, Murali *et al*., 2007). *Terminalia bellerica* (commonly *Bahera*) is extensively used in various diseases as an antispasmodic, bronchodilator, hepatoprotective and antiasthmatic (Jadon *et al*., 2007, Gilani *et al*., 2008). Its hypoglycemic property was also observed in alloxan induced diabetic rats (Sabu and Kuttan, 2002). In *Ayurveda* the analysis of the nature and property of the medicine is based on their elemental composition (*dravya*), quality (*guna*), taste (*rasa*), potency (*virya*), Metabolic action (*vipaka*), effect (*karma*) and their specific potency (*prabhava*):

Dravye Raso Guno Virya Vipakah Shaktirevach Padarthah Panch Thishthanti Swam Swam Kurvanti Karma Cha (Jln, 2007).

The aim of the present study is to develop a polyherbal formulation *Diabcap* (containing medicinal plants in Table 1) and evaluate its antidiabetic and hepatoprotective activity in SIDRs. Relying and respecting the holistic approach of *Ayurveda* the plant materials in *Diabcap* was selected on above mentioned physicochemical and pharmacological attributes.

Preparation of polyherbal drug formulation: All the six plants were washed in running water, then dried, ground to powder and mixed in a definite ratio in geometrical manner. The mixture was suspended in distilled water (50 g powder per 400 ml water) and heated under reflux for 30 min, after which the decoction was centrifuged, filtered and concentrated using rota vapour (Buchi R-210). It was frozen at −20 °C and then lyophilised. The crude yield of the lyophilised material was approximately 16.23 % (w/w); it was stored at −20 °C until further use.

Standardization of *Diabcap*

Formulation was identified as per WHO guidelines. Quantitative analysis of raw material was carried out for organic matter, extractive values (water soluble and methanol soluble), total ash, and acid-insoluble ash. Moisture content and pH were also assessed. An HPLC fingerprint profile was also performed using isocratic elution of water-acetonitrile (85:15) at 0.5ml/min as mobile phase.

Animal care and selection

Adult albino rats (Charles Foster Strain) weighing about 150-180gm were used. All rats were given a period of acclimatization for 1 week before starting the experiment under 12 hour light/dark cycle. They were fed ad libitum with standard chow diet. Institutional Animal Ethics Committee (IAEC), constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, approved the experimental protocol.

Acute oral toxicity study

The acute oral toxicity of *Diabcap* in adult rats was studied as per OECD guideline 423. Female albino rats (230 ± 20gm) were divided into two groups (n=6). Group I served as control and received water as vehicle and Group II received *Diabcap* (2000mg/kg, orally) prepared in water. Mortality, clinical and other gross pathological signs were recorded and it was found that the formulation was safe up to the dose of 2 g/Kg BW for rats.

Table 1. Targeted plant parts in Polyherbal preparation

Common name (Hindi name)	Botanical name	Part used	Family
Indian barberry (*Daru haridra*)	*Berberis aristata*	Root	Berberidaceae
Nut Grass (*Nagarmotha*)	*Cyperus rotundus*	Rhizome	Cyperaceae
Cedar wood (*Dev daru*)	*Cedrus deodara*	Heartwood	Pinaceae
Indian gooseberry (*Amla*)	*Emblica officinalis*	Fruit	Phyllanthaceae
Chebulic myrobalan (*Harad*)	*Terminalia chebula*	Fruit	Combretaceae
Beleric Myrobalan (*Bahera*)	*Terminalia bellerica*	Fruit	Combretaceae

METERIALS AND METHODS

Chemicals and reagents: STZ purchased from Sigma Chemical Co. (St. Louis, MO, USA). Biochemical kits were purchased from Merck (Darmstadt, Germany). All the other chemicals were of analytical grade and purchased from Hi-media (India). Only distilled water was employed for preparing the reagents.

Plant material: The mentioned parts of the plants used were collected locally and from the research farm of the CSIR-CIMAP and authenticated by the Department of Botany, CSIR-CIMAP, Lucknow. A voucher specimen (HMPD/2010 Di-201) has been preserved at Herbal Medicinal Product Department CSIR-CIMAP CIMAP for future reference.

Sub-acute oral toxicity study

The rats were distributed into four group of 6 animals each: Group I received distilled water and served as control while the other four groups were treated with aqueous suspension of *Diabcap* at 5, 50 and 300mg/Kg BW once orally for 28 days. Body weight was measured weekly during the experimental period. Clinical observations, behavioral profile, autonomic activity (salivation, pupil size or any contortion), nervous activity (posture, exploratory movement), mortality and any sign of ill health were performed daily. Blood samples for hematological and clinical chemistry examination were studied at the end of the experimental period in fasting condition. Animals were sacrificed and all organs were carefully examined. Present study was performed according to the

"Guidelines for Repeated Dose 28-Day Oral Toxicity Study in Rodents (1995; No. 407)"

Experimental design and dose optimization

To evaluate the glycaemic potential, initial screening of *Diabcap* was done by conducting oral glucose tolerance test (OGTT) following the method of Jaiswal *et al*. (2009). Generally 1/5th to 1/10th of the lethal dose is chosen for effective dose calculation (Naskar *et al*., 2011). Therefore, 300 and 500 mg/kg BW doses were chosen in the study. Then the antidiabetic effect of extract was assessed in SIDRs with a dose identified as the effective dose by initial screening.

Induction of diabetes: Blood glucose was determined after an overnight fast with free access to water. On the following day, diabetes was induced by single intraperitoneal administration of STZ, freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 60mg/kg BW (Sachdewa and Khemani, 2003, El-Hilaly *et al*., 2006). The control rats received the same amount of 0.1M citrate buffer. Fasting blood glucose was checked before and after 72hrs post administration with the help of glucometer using strip method and only those animals which showed hyperglycaemia (blood glucose levels ≥ 200–500 mg/dl), were considered diabetic and were included in study.

Assessment of antidiabetic activity in SIDRs

Animals were weighted before the experiment and divided into following five groups of 6 rats each. The duration of experiment was 21 days from STZ treatment and 17 days from *Diabcap* administration.

Group I(Control group) given distilled water during the experimental period.

Group II (diabetic rats as negative control) given distilled water/day.

Group III (diabetic rats as Positive Control) were given Metformin (Mfn) orally at a dose of 500 mg/Kg BW/day on and from the 4th day for 17 days (Pushparaj *et al*., 2007).

Group IV (diabetic rats treated with *Diabcap*) animals were given *Diabcap* orally at a dose of 300 mg/Kg BW/day on and from the 4th day for 17days.

Group V (diabetic rats treated with *Diabcap*) animals were given *Diabcap* orally at a dose of 500 mg/Kg BW/day on and from the 4th day for 17 days.

After 21 days of oral treatment, experiment was terminated and observations were made. BW was taken with the help of single pan balance. Fasting Blood glucose was estimated on every 8th day interval with the help of Glucometer (Ascensia Entrust from Bayer) using strip method and blood was taken from tip of the tail. On the 21st day all animals were sacrificed by decapitation.

Blood sampling and biochemical analysis

Blood was collected from orbital plexus on 21st day into a centrifuge tubes and allowed to clot for 30 min at room temperature then the samples were centrifuged at 3000 rpm for 10 min, serum was separated and stored at -20 ^{0}C until analysis was done and then animals were sacrificed. Serum samples were analyzed spectrophotometrically for estimation of biochemical parameters using analytical kits.

Histopathology of liver

After decapitation liver samples were removed quickly and fixed in 10% formalin for histopathological examination. After complete fixation slices of samples (3-4mm) were dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin. 5µm thick sections were obtained washed with xylene (2min each) and mounted on glass slides treated with Dibutyl phathalate xylene (DPX). Then sections were stained with hematoxylin–eosin stain for microscopic examination.

Statistical analysis: Results were expressed as Mean ± SEM and compared by ANOVA. Statistically significant difference was calculated using tukey's test. Statistical significance was set at $P < 0.05$.

RESULTS

Standardization of *Diabcap*

HPLC Fingerprint of aqueous extract of *Diabcap* and its components are shown in Figure 1. 5 peaks with retention time 2.172, 2.964, 4.067, 4.293, 4.511 were identified as the characteristic fingerprints of *Diabcap*. This HPLC fingerprint will help in maintaining the quality and batch to batch consistency of *Diabcap*. The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value is an indicative of silicate impurities. The loss on drying value indicates the amount of moisture content present in the drug. The extractive values indicate the amount of active constituent in given amount of plant material when extracted with respective solvent. The values obtained were within the limits mentioned in the Ayurvedic Pharmacopoeia of India and WHO (Table 2).

Table 2. Evaluation of extract

Parameter	Standard Value	*Diabcap* (Obtained value)
Physical tests		
a. Nature		Powder
b. Color		Dark brown
c. Odor		Characteristic
d. Taste		Bitter
Moisture content (%)	≤ 10	8.9
Ash values (%)		
a. Total ash	7-11	6.004463
b. Acid insoluble ash	≤ 2	0.569783
Extractable Matter (%)		
a. Alcohol soluble extractive	15-20	11.15
b. Water soluble extractive	10-20	8.67

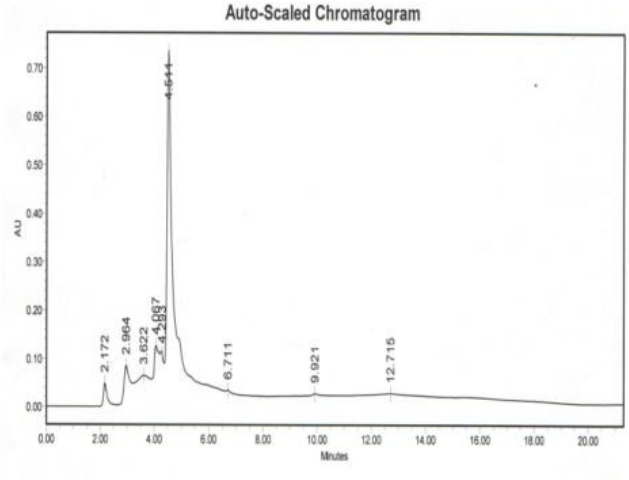


Fig. 1. HPLC Fingerprint of antidiabetic formulation *Diabcap*

Table 3. Serum biochemical findings in rats treated with *Diabcap* for treatment (28-days) period. Parameters (mean ± SEM)

Param-eters	0th Day				14th Day				28th Day			
	Control	5mg/Kg BW	50mg/Kg BW	300mg/Kg BW	Control	5mg/Kg BW	50mg/Kg BW	300mg/Kg BW	Control	5mg/Kg BW	50mg/Kg BW	300mg/Kg BW
AST	38.72±1.97	34.25±1.36	34.61±1.41	33.64±1.65	34.20±1.83	35.35±1.09	36.15±1.72	31.96±2.63	38.35±1.51	42.81±1.86	42.46±1.59	40.78±1.12
ALT	25.09±1.24	25.94±1.42	27.41±1.17	25.67±1.04	25.38±1.6	27.62±1.69	26.45±2.08	27.97±2.01	29.68±2.1	31.61±1.12	33.00 ±0.95	34.99 ±1.73
ALP	64.15±1.49	60.81±1.76	60.71 ±1.14	61.76 ±1.85	62.89 ±1.38	63.85±1.9	61.47 ±1.41	60.17 ±1.79	66.12 ±0.89	64.01 ±1.97	64.63 ±2.06	62.29 ±1.53
TC	103.43±1.89	108.38±1.92	109.16±1.02	104.3±2.25	88.24±2.65	88.04±3.30	94.88±3.33	98.56±2.33	99.05±2.66	101.41±1.97	97.53±2.67	102.70±1.84
TG	60.93±3.56	69.04±1.64	53.38±7.69	68.87±3.38	45.83±9.07	48.66±5.37	42.9±11.31	53.80±4.77	54.65±7.05	62.51±2.88	61.40±9.30	62.86±8.27
HDL	33.11±3.88	30.36±6.46	31.62±4.47	25.98±5.09	33.60±5.77	32.45±3.73	24.45±5.89	34.35±6.20	41.99±5.83	39.29±5.53	31.55±3.04	48.22±4.69
CRE	0.31± 0.13	0.13± 0.03	0.31±0.11	0.35±0.10	0.31±0.07	0.25±0.09	0.31±0.07	0.27±0.05	0.37±0.04	0.46± 0.08	0.4± 0.05	0.54± 0.04
BUN	18.46 ± 2.14	17.41±3.34	20.44±5.06	22.67±6.02	19.57±4.59	21.20±4.60	21.31±4.23	22.63 ±6.17	16.09±2.53	15.83±1.11	20.23±2.72	20.47±4.28

Values are mean ± SEM from six animals in each group. Non-significant changes were observed as compared to control.

OGTT of normal healthy rats

Figure 2 indicated the hypoglycemic effect of a single oral administration of two doses. The dose of 300mg/Kg produced a maximum fall of 32.8%, whereas a fall of 33 and 34.8% was observed with metformin and 500mg/ Kg groups respectively.

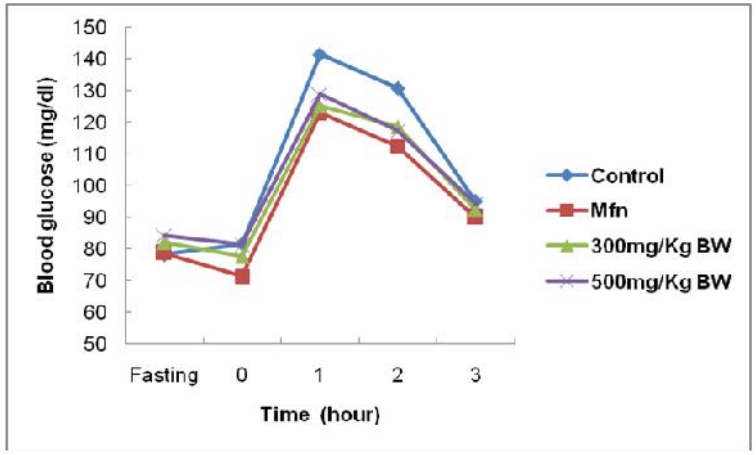


Fig. 2. Hypoglycaemic effect of Diabcap in OGTT; each value shown is mean ± SEM. (n = 6)

Acute oral toxicity study

Diabcap did not exhibit any toxic symptoms up to the dose level of 2000mg/Kg BW therefore further dosing to estimate the LD50 was not performed (data not shown). According to OECD guidelines for acute toxicity, an LD50 dose of 2,000 mg/kg and above is categorized as unclassified, and hence, the drug is found to be safe (WHO 1993).

Sub-acute oral toxicity study

During the experimental period, the three doses of *Diabcap* showed no signs of toxic effects or mortality, nor were any significant clinically relevant changes observed in general behaviour or physiological activities. There was no significant difference in bodyweight of treated animals compared to controls. The biochemical analyses are shown in Table 3. The haematology parameters measured after 28 days remained within physiological ranges (Fig. 3) and treatment for 28 days did not cause any statistically significant changes in serum glucose, total cholesterol, triglycerides, urea, or in the activity of the marker enzymes of liver injury (AST, ALT). Though repeated oral dosing produced an increase in liver weight than control in treatment groups (Fig. 4). A possible explanation for the increased liver weight could be the induction of hepatic detoxification enzymes such as cytochrome P450,(Yokotani *et al*., 2013) a mechanism currently being investigated.

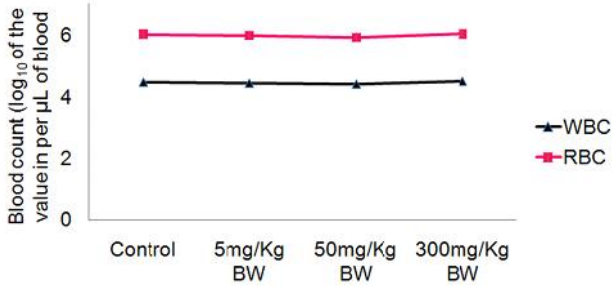
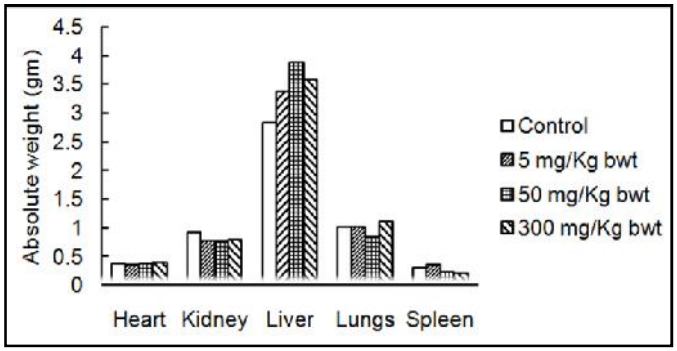


Fig. 3. Total RBC and WBC count/µL blood in albino rats after 28 days sub-acute oral administration of *Diabcap* (values are represented in $\log base_{10}$ of the value in per µL of blood); Non-significant changes were observed as compared to control; each value shown in mean ± SEM (*n* = 6).

Body weight and Blood glucose in SIDRs- SIDRs significantly ($P < 0.001$) decreased their body weight when compared with the rats of normal control group. A normal body weight gain was observed in normal control group at the end of 21st day. The treated diabetic rats did not showed any sign of recovery in BW when compared to diabetic untreated group (Table 4).



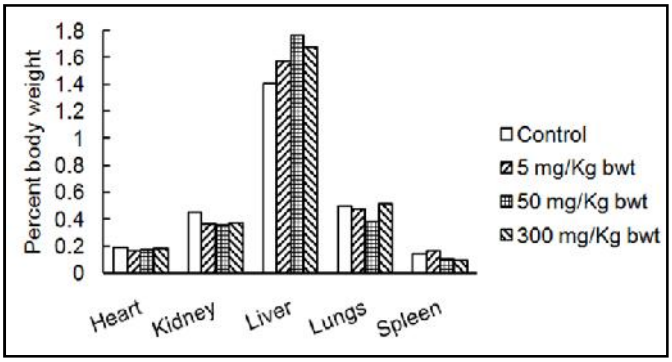


Fig.4. Effect of *Diabcap* on differential absolute and relative organ weight in albino rats. Non-significant changes were observed as compared to control; each value shown in mean ± SEM (*n* = 6)

Table 4. Effect of Polyherbal preparation on body weight in normal and SIDRs

Groups	day 0	day 7	day 14
Normal Control	176.8 ± 8.5	183.6 ± 7.5	196.2± 10.0
Diabetic Control	173.8 ± 8.2	165.2 ± 8.5	113.8 ± 10.1***
Diabetic (Mfn)	172.4 ± 8.6	164.2 ± 9.4	129.6 ± 8.4***
Diabetic (300mg/Kg)	169 ± 7.8	161.4 ± 6.6	135.2 ± 10.9***
Diabetic (500 mg/Kg)	171.8 ± 5.8	164.2 ± 5.8	131.2 ± 5.0***

Values are mean ± SEM from six animals in each group. *** P < 0.001: as compared to normal control on corresponding day.

The blood glucose is significantly increased (P < 0.001) after STZ as compared to normal rats. Thereafter a significant decrease of blood glucose was observed (P < 0.001) with Mfn and 300mg/Kg dosing groups. On the contrary in 500mg/Kg dosing group, no significant change compared to that of diabetic control was detected (Table 5).

Liver function test (serum levels of AST, ALT, ALP and bilirubin): There was a significant increase (P<0.001) in the level of AST, ALT and ALP in SIDRs as compared to normal control. After twenty one days levels of AST was found to be significantly decreased with 300mg/Kg, and Mfn dosing groups as compared to the diabetic control group. Whereas 500mg/Kg dosing group reduced the ALT level but not the AST level as compared to diabetic control. Similarly after treatment bilirubin level was significantly decreased with 300mg/Kg, 500mg/Kg and Mfn dosing groups as compared to the diabetic control group, whereas no change was found with ALP level in any of the treatment group (Table 6).

Serum levels of Cholesterol and triglyceride: Total serum cholesterol as well as triglyceride increased significantly (P<0.001) in diabetic rats as compared to normal rats. Whereas treated SIDRs showed significant decrease (P<0.001) in total cholesterol with 300mg/Kg ,500mg/Kg and Mfn dosing groups as compared to the diabetic control group. In the same way triglyceride reduced significantly (P<0.05) with 500 mg and Mfn group and in 300mg dosing group at P< 0.01 (Table 7).

Table 7. Effect of Polyherbal preparation on Total cholesterol and Triglyceride

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
Normal Control	134.9 ± 0.77	52.93 ± 1.41
Diabetic Control	282.5 ± 2.5***	91.79 ± 11.74***
Diabetic (Mfn)	166 ± 4.3***	65.3 ± 1.9*
Diabetic (300mg/Kg BW)	213.9 ± 2.5***	57.8 ± 1.5**
Diabetic (500 mg/Kg BW)	250.5 ± 2.64***	67.7 ± 1.7*

Values are mean ± SEM from six animals in each group. * P < 0.05; **P < 0.01;***P<0.001: diabetic control was compared with normal control and treated groups were compared with diabetic control on the corresponding days.

Histopathological Study

Pathologically, liver tissues showed normal arrangement of hepatocytes with no visible lesions. On the contrary liver tissues in SIDRs showed increase in connective tissue, degeneration of hepatocytes with numerous vacuolations, dilated blood vessels showing congestion and necrosis. Treated groups with Mfn and *Diabcap* prevented the pathologic changes and showed more significant normalized tissue in comparison to diabetic (Fig. 5).

Table 5. Effect of Polyherbal preparation on blood glucose(mg/dl) in normal and SIDRs

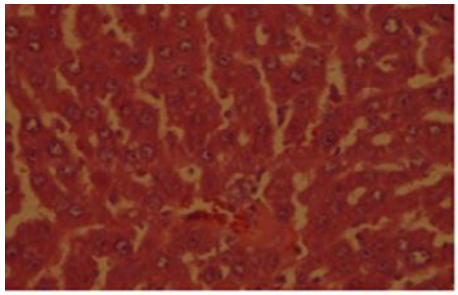
Groups	Blood glucose ($mgdl^{-1}$)			
	Before treatment (initial)	Day 4	Day 12	Day 21
Normal Control	88.2 ± 2.55	92.2 ± 4.5	95.2 ± 1.5	111.6 ± 3.14
Diabetic Control	92.2 ± 1.46	389.8 ± 31.9	453.2 ± 2.7	374.8±18.315***
Diabetic (Mfn)	87 ± 2.02	429.8 ± 1.5	356.4 ± 3.6	181.8 ± 1.6***
Diabetic (300mg/Kg)	96.6 ± 2.9	424 ± 13.2	252 ± 4.1	180.8 ± 3.9***
Diabetic (500 mg/Kg)	85 ± 2.4	491.4 ± 2	443 ± 3.5	328.6 ± 4.2

Values are mean ± SEM from six animals in each group. ***P<0.001: diabetic control was compared with normal control and treated groups were compared with diabetic control on the corresponding days.

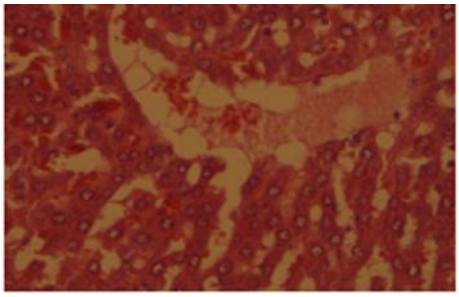
Table 6. Effect of Polyherbal preparation on liver function tests

Groups	AST (IU/L)	ALT (IU/L)	ALP(IU/L)	Bilirubin (mg/dl)
Normal Control	19.27 ± 0.6	12.99 ± 0.2	129.1 ± 0.9	0.14 ± 0.008
Diabetic Control	31.21 ± 0.28***	26.24 ± 0.3***	241.8 ± 1.2***	6.5 ± 0.28***
Diabetic (Mfn)	28.3 ± 0.25***	13.5 ± 0.3***	314.7 ± 1.1	0.73 ± 0.12***
Diabetic (300mg/Kg)	18.41 ± 0.23***	20.6 ± 1.9***	182.5 ± 1.6	0.14 ± 0.02***
Diabetic (500mg/Kg)	30.23 ± 0.18	11.9 ± 0.4***	211.4 ± 0.9	0.44 ± 0.03***

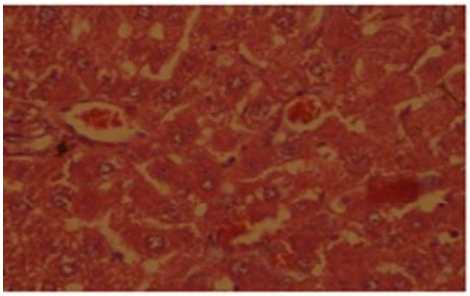
Values are mean ± SEM from six animals in each group. ***P<0.001: diabetic control was compared with normal control and treated groups were compared with diabetic control on the corresponding days.



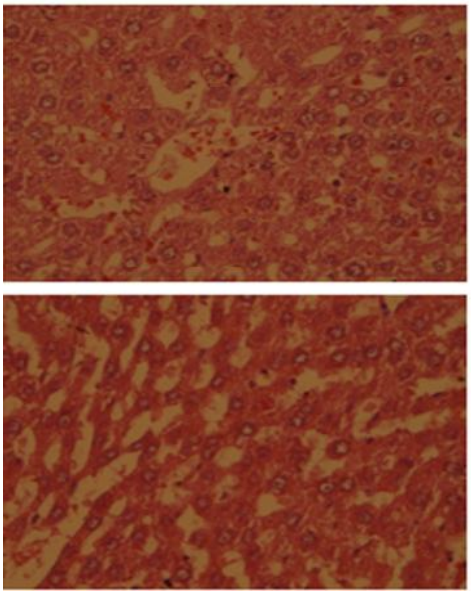
a. Normal control rat showing normal hepatic structure



b. Tissue of streptozotocin diabetic rat showing increase in the connective tissue, degeneration of hepatocytes with numerous vacuolations;blood vessels showing congestion and necrosis



c. Metformin treated with 500 mg/kg BW showing normalization of tissue



d and e, *Diabcap*-treated groups with 300, 500mg/kg BW, respectively, showing normalization of the tissue

Fig. 5. Effect of *Diabcap* on 5 micron thick section of liver in streptozotocin-induced diabetic rats

DISCUSSION

According to the recent reports of WHO approximately 90% of people have T2DM among the 347 million people worldwide who have diabetes (Sealand *et al*., 2013). Type-2 diabetes mellitus (T2DM) is now well recognized as one of the silent killer disease claiming many lives world over. Elevated blood sugar puts a strain on almost every organ and other parts of the body and place the patient to a higher risk of micro and macro-vascular complications. Many hypoglycaemic agents have been used in the disease management but are often associated with serious side effects. Consequently, attention has been focused on the use of plants and herbal remedies as alternatives in the treatment of diabetes mellitus because they are believed to be safer and devoid of adverse effects (Wais *et al*., 2012). In comparison with individual herbs, compound formulae exhibit greater efficiency and lower toxicity. The therapeutic potencies of herbs are found additive, or even synergistic, when used in combination. In polyherbal formulations plants are selected in such a way that it exhibits the desired activity and the undesired activities will be diluted or absent altogether (Jagetia *et al*., 2004). In the Type-2 diabetes mellitus (T2DM), all the plants used in the *Diabcap* have been experimentally documented to possess antidiabetic potential individually. In the present study, data obtained clearly indicate that the oral administration of *Diabcap* exhibited a good anti-hyperglycemic effect. Anti-diabetic activity of *Diabcap* at the dose of 300mg/Kg is comparable to Metformin at 500mg/Kg dose. The effect of the polyherbal mixture is may be due to the presence of more than one anti-hyperglycemic principle and their synergistic properties. Changes of body weight in diabetic and normal rats varied. Since the normal rats are in the growing age, they showed a slight weight gain as diabetic loss of weight is not seen in them. All diabetic rats showed a progressive fall in body weight throughout the experimental period. The untreated as well as the treated SIDRs drank a large amount of water and undergo frequent urination. In acute toxicity studies, *Diabcap* did not show any symptoms of toxicity or mortality when dosed up to 2000 mg/kg. In the present study, significant elevations in the biochemical markers of liver injury (ALT, AST, ALP and Bilirubin) imitate the hepatocytes injury in experimental diabetes. This is consistent with the findings of abolfathi *et al*. (Abolfathi *et al*., 2012). Marked elevations in serum aminotransferases and characteristic histological changes in liver tissues were used as a diagnostic of hepatopathy. (Demirci *et al*., 2012) The most common liver function test (LFT) include the serum aminotransferases, alkaline phosphatase, serum bilirubin, serum albumin. Their increased level indicates the abnormalities in liver or in bile duct. For instance elevated aminotransferase typically indicates hepatocellular injury. (Aragon and Younossi, 2010) Treatment with Diabcap normalized the activity of these enzymes, indicating its potential in inhibiting liver damage induced by STZ. Lipid abnormalities are common in DM. The characteristic disturbance in type-2 diabetes is called diabetic dyslipidemia which consists of elevated VLDL (Very low density lipoprotein) and triglyceride, low HDL (High density lipoprotein). (Williams *et al*., 2004) Hypertriglyceridemia and hypercholesterolemia are most common lipid abnormalities in diabetes. (Sachdewa and Khemani, 2003) In the present study serum cholesterol and triglyceride level increases significantly in SIDRs compared to normal control, however repeated administration of the preparation significantly lowered these serum values. The present study also investigated the dose related oral acute and repeated dose toxicity of *Diabcap* on organs, tissues, blood parameters, and blood chemistry, using

rats and thus provides the experimental evidence of the safety of the formulation. Employing the sensitivity of hematopoietic system to toxic substances, analysis of hematological parameters indicated the nontoxic behavior of *Diabcap*. (Barcellona *et al*., 2012).

Conclusion

The current scenario warrants a systematic study on herbal medicine as it is critical to comprehensively evaluate the efficacy as well as the safety profile of herbal formulae. Significant prevalence of hepatic disorders in relation to recent onset of T2DM compels us to divert our vision and therefore the study was designed to target the hyperglycemia together with glycemic hepatopathy. *Diabcap* possesses antidiabetic effect on SIDRs. Most likely it exerts multiple effects including both on serum liver function and lipid levels. Further study can be undertaken to elucidate the exact mechanism and to isolate and identify its active principle(s). The study supports that *Ayurvedic crude drugs* could be a better starting material to find "new leads" for management of DM.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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