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Research Article

FRACTIONATION AND EVALUATION OF ANTIDIABETIC, ANTIHYPERLIPIDEMIC, AND ANTIOXIDANT ACTIVITY OF *DECALEPIS HAMILTONII* AND *SHOREA TUMBUGGAIA*

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ABSTRACT

The objective of study was to evaluate antidiabetic, hypolipidemic, and antioxidant activities of the fractions of the roots of *Decalepis hamiltonii* (DH) and leaves of *Shorea tumbuggaia* (ST) [1] in experimentally induced diabetic rats. Alloxan (150 mg/kg), intraperitoneal) was used to induce hyperglycemia. Administration of different fractions at different doses once daily up to 28 days to diabetic rats reduced blood glucose and glycosylated hemoglobin [2], and increased insulin levels significantly. Triglycerides, total cholesterol, very low density lipoproteins and low density lipoproteins levels were significantly decreased, whereas, high density lipoproteins levels were increased. The fractions also significantly reduced reactive thiobarbituric acid levels (thiobarbituric acid reactive substances), super oxide dismutase and increased the levels of reduced glutathione and catalase when compared to the diabetic-control animals. DH and ST were assessed for *in-vitro* antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide and reducing power model. Significant *in-vitro* antioxidant activity was observed in all the models. From the results, it is evident that alcoholic fractions of the roots of DH can be effectively used as antidiabetic [3], hypolipidemic, and antioxidant activity.

Keywords: Antidiabetic, Antioxidant, Hypolipidemic, Fractions, *Decalepis hamiltonii.*

INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism due to deficient action of insulin on target tissues resulting from defects in insulin secretion, insulin action or both [10]. It has been suggested that a total of 300 million people around the world will have diabetes by the year 2025 and the global cost of treating diabetes and its complications could reach US $1 trillion annually. The high prevalence and severity of the disease is quite alarming in most of the developing countries [35]. In India alone has more than 40 million diabetic individuals, which represent nearly 20% of the total diabetes population of the world and it is estimated to rise to almost 70 million by 2025. Although different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, none offers complete glycemic control [18]. There is increasing demand by the patients to use the herbal preparations with antidiabetic activity.

Decalepis hamiltonii (DH) Wight and Arn, [4] commonly called as maredu kommalu or baree sugandhi or makali beru is an endangered climbing shrub belonging to the family asclepiadaceae. Its roots have been used in ayurveda, to stimulate appetite, relive flatulence and as a general tonic, [28]demulcent, diaphoretic, diuretic, and tonic. It is useful in the loss of appetite, fever, skin disease, diarrhea, nutrition disorders, blood purifier, flavoring principle. ST is an endemic and globally endangered semi-evergreen tree species restricted to the southern Eastern Ghats up to 1000 m, distributed in Seshachalam and Veligonda hills in Cuddaph, Tirupati hills in Chittoor district, Andhra Pradesh. North Arcot and Chengalpattu district, Tamil Nadu. It is more prevalent at drier areas in non-teak mixed deciduous forest vegetation at an altitude of 300 m amsl [32]. The tree trunk is used as flagpoles for temples. The stem is source of resin, which is used as incense and as a substitute in marine yards for pitch. It is also used in indigenous medicine as an external stimulant and a substitute for abietis; resina and Pix Burgundica of European Pharmacopoeias. The plant extracts are used as a cure for ear-aches and leaf juice is used as ear drops for children. Methanolic extract of leaves of this plant reported to have antinociceptive and anti-inflammatory activity. Stem bark is reported to have antiulcer activity.

Methanolic extract of leaves of this plant reported the phytochemical constituents present in DH roots are ellagic acid, volatile oil (0.68%), which contain 2-hydroxy, 4-methoxy benzoic acid (96%), salicyaldehyde (0.018), benzaldehyde (0.017%), methylsalicylate (0.044%), benzyl alcohol (0.016%), 2-phenyl ethyl alcohol (0.081%), ethyl salicylate (0.038%), p-anisaldehyde (0.01%), and vanillin (0.45%), ketone, resinol, sterols, saponins, tannins, inositol, fatty acids, α-amyrin, β-amyrin acetate, and lupeol. administration of 2-hydroxy 4-methoxy benzoic acid, is beneficial in normalizing the altered carbohydrate and lipid metabolism in diabetes, and also protects the liver by restoring the levels of liver specific enzymes [27]. There are no systemic pharmacological studies to establish the antidiabetic effect of DH (EDH). Hence, the present investigation was carried out to study the antidiabetic, hypolipidemic, and antioxidant activities fractions of DH (EDH) in alloxan-induced diabetic rats. The alcoholic extract of *Shorea tumbuggaia* (EST) was screened for various phytoconstituents such as steroids, alkaloids, tannins, flavonoids, and glycosides by employing standard phytochemical tests.

MATERIALS AND METHODS

Plant material and fractionation

DH roots and ST leaves are freshly collected during the month of August-September from the forest area Dhulepalli, Hyderabad. The Plant material was authenticated by Dr. V.S. Raju, Department of Botany, Kakatiya University, and Warangal. Voucher specimen was deposited at Vaagdevi College of Pharmacy, Warangal. The plant material was shade dried, powdered, and material was fractionized with ethanol (0.5 kg powder and 2 L 95% ethanol) by reflexing over a boiling water bath for 4 hrs. The fractions were dried under reduced pressure using a rotary vacuum evaporator. The % yield was 4.2% w/w and the fractions were kept in refrigerator for further use.

Drugs and chemicals
Alloxan was purchased from Sigma Chemicals, USA. Standard drug glibenclamide was procured from Cipla Ltd. Diagnostic kits used in this source from Span Diagnostics Ltd. India. All the other chemicals used were of analytical grade.

Animals
Wistar albino rats weighting 120-180 g were procured from Mahaveer Enterprises, Hyderabad. (CPCSEA Regd. No: 146/1999/CPCSEA). They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature, and relative humidity. Animal experiments were designed and conducted in accordance with the guidelines of Institutional animal Ethical Committee (IAEC-VCOP, Warangal) (1047/ac/07/CPCSEA, dated 13-06-2009).

Acute toxicity studies
The oral acute toxicity study of the plant fractions was carried out in adult Swiss albino mice of both sexes. This method was carried out according to OECD guidelines by adopting fixed dose method. Four animals per treatment group and different dose range 5, 50, 300, and 2000 mg/kg respectively, the animals were observed continuously for any change in autonomic or behavioral response for first 2 hrs, then intermittently and at the end of 24 hrs, the mortality/survivor was recorded [34].

Oral glucose tolerance test (OGTT)
Rats were fasted overnight and divided into different groups with 6 animals in each group.

Group I received distilled water, served as control.
Group II animals were treated with glibenclamide (0.5 mg/kg p.o.) to serve as standard.
Group III onward animals were treated with DH, ST in different fractions doses.

The groups control, standard, and test were treated with drugs 30 minutes prior to the glucose load (2 g/kg p.o.) [21]. Blood samples were collected at 30, 60, 90, and 120 minutes after glucose loading, by retro orbital venous puncture and glucose levels were measured immediately after separation of serum.

Experimental induction of diabetes in rats
Alloxan monohydrate was used to induce diabetes. Animals were allowed to fast for 16 hr and were injected intraperitoneally (i.p.) with freshly prepared alloxan monohydrate in normal saline in a dose of 150 mg/kg. After 48 hrs, the rats which have blood glucose levels of 200 mg/dl and above were considered to be diabetic.

Experimental design
After induction of diabetic the rats were divided into different groups of six animals each.

Group I : Normal rats.
Group II : Diabetic rats treated with the vehicle solution (2% gum acacia ml/kg, *os*).
Group III : Diabetic rats treated with glibenclamide (5 mg/kg, *os*).
Group IV : Diabetic rats treated with petroleum ether fractions of DH (PEFDH) and ST (PEFST).
Group V : Diabetic rats treated with ethyl acetate fractions of DH (EAFDH) and ST (EAFST).
Group VI : Diabetic rats treated with chloroform fractions of DH (CFDH) and ST (CFST).
Group VII : Diabetic rats treated with aqueous fractions of DH (AQFDH) and ST (AQFST).

The drugs and vehicle were administered orally by an intragastric tube daily for 28 days. The body weight was measured on days 7, 14, 21, and 28, and blood samples were drawn from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after they had been fasted for 12 hrs. The blood samples were used for the biochemical analysis of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), very low density lipoproteins (VLDL), and high density lipoproteins (HDL).

Bio-chemical analysis
The blood samples were centrifuged at 5000 rpm for 20 until analysis was done. Samples were analyzed spectrophotometrically for blood glucose by glucose oxidase-peroxidase (POD) method, using commercial kit (Span diagnostics, India). TG was estimated by glycerol-3-phosphate oxidase-POD method, TC [15] was estimated by cholesterol oxidase-phenol 4-aminoantipyrine peroxidase method, HDL - was analyzed by kits (Roche diagnostics, Germany) LDL, and VLDL-cholesterol using Friedevald's equation (Roche diagnostics, Germany). Glycosolated hemoglobin by Gould *et al.,* 1982 and serum insulin were estimated by ACS: 180 automated chemiluminescence system. On 28th day, serum glycosylated hemoglobin (HbA1c) and insulin estimations were carried out at Vijaya Diagnostic Center, Hanamkonda, Andhra Pradesh.

***In-vitro* antioxidant activity**

Estimation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The hydrogen atom of electron donating abilities of the resultant compounds was measured from the bleaching of the purple-colored ethanol-solution of DPPH. This Spectrophotometric assay uses the stable radical DPPH as a reagent [11]. 0.1 mM solution of DPPH in ethanol was prepared and 0.1 ml of this solution was added to 3 ml of different concentration of the fractions (5, 10, 20, 40, 60, 80, and 100 µg/ml). After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activity of the fractions was expressed as IC_{50}. The IC_{50} value was defined as the concentration (µg/ml) of fractions that inhibits the formation of DPPH radicals by 50%.

Estimation by reducing power method

The reducing power of the compound was evaluated. Different concentrations of the fractions (5-100 µg/ml) were dissolved in distilled water and added with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% of $K_3Fe(CN)_6$. The mixture was incubated at 500 c for 20 minutes. 2.5 ml of 10% trichloro acetic acid (TCA) was added to the blend and centrifuged at 3000 rpm for 10 minute. The upper layer of the solution (2.5 ml) was assorted with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated reducing power.

Estimation by hydroxyl radical scavenging activity

The scavenging ability for hydroxyl radical was measured. Stock solution of ethylenediaminetetraacetic acid (EDTA) (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM), and deoxyribose (10 mM) were prepared in distilled de-ionized water. The assay was preformed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxyribose, 1 ml of different concentrations of fractions (5-100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid added in sequence. The mixture was then incubated at 37°C for 1 hr. A 1.0 ml of incubated mixture was mixed with 1 ml of 10% TCA and 1 ml of 0.5% thio-barbituric acid (TBA) in 0.025 M NaOH containing 0.025% butylated hydroxyl anisole to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the fractions is reported as % incubation of deoxy ribose degradation.

I % = (A blank–A sample/A blank) × 100

***In-vivo* antioxidant studies**

Preparation of liver post mitochondrial supernatant (liver-PMS)

At the end of the study, animals were decapitated and cut open to excise the liver. The excised livers immediately and thoroughly washed with ice-cold physiological saline. The tissue of 100 mg was homogenized in 1 ml of 0.1 M cold tris-HCl buffer (pH7.4) in a Potter-Elvehjam homogenizer fitted with a Teflon plunger at 600 rpm for 30 minute [13]. The homogenate was centrifuged at 10,000 g for 20 minute at 40c and the supernatant with firmly packed pellets was resuspeneded by homogenization in 100 mM Tri-Hcl buffer containing 20%w/v glycerol and 0.1 ml of 10 mM EDTA, pH 7.4. The PMS was used to assay thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity.

Estimation of lipid-peroxidation (LPO) from liver PMS

LPO was induced and assayed in rat hepatic-PMS. In 1 ml of the reaction muddle, 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml of hepatic PMS (10%w/v), 0.2 ml ascorbic acid (100 mM) and 0.02 ml ferric chloride (100 mM) and was incubated at 37°C in a shaking water bath for 1 h. The reaction was clogged by the addition of 1 ml TCA (10%, w/v),

Table 1: Effect of fractions of DH on blood glucose levels in alloxan-induced diabetic rats

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours after the treatment						
		0 hr	2 hr	4 hr	6 hr	8 hr	16 hr	24 hr
Normal (I)	-	88.5±5.9	87.2±6.05 (1.4±0.4)	84.7±7.3 (4.3±0.5)	82.3±6.3 (7.02±1.5)	83.01±4.6 (6.1±1.7)	83.4±4.7 (5.6±1.5)	82.5±5 (6.7±0.1)
Diabetic control (II)	-	281.9±9.7	279.1±11.2 (1.0±0.6)	276.8±10.3 (1.7±1.2)	268.1±10.4 (4.7±3.2)	264.8±22.5 (6.1±3.7)	268.0±17.1 (5.1±3.7)	271.1±9.41 (3.9±2.5)
Glibenclamide (III)	5	265.95±11.9	249.3±9.4 (6.1±2.3)	217.69±14.0** (18.0±5.5)	175.79±18.6** (33.9±6.8)	189.3±17.1** (28.7±6.5)	242±14.7 (9.0±4.6)	257±9.2 (3.3±2.1)
PEFDH (III D)	60	270.9±13.8	259.4±13.8 (4.2±1.3)	250.4±12.9 (7.5±1.6)	238.1±11.6 (12.0±2.3)	246.1±12.2 (16.2±3.6)	267.6±9.0 (1.0±5.4)	279.5±6.9 (−3.4±5.7)
PEFDH (IV D)	120	285.6±13.4	271.8±12.1 (4.8±0.8)	254.9±10.3 (10.6±2.3)	245.2±12.7 (14.1±2.9)	257.7±15.6 (10.1±4.8)	268.1±5.9 (6.0±3.2)	279.2±3.9 (2.1±3.9)
CFDH (V D)	25	291.2±8.7	281.3±8.4 (3.4±1.7)	262.3±5.8 (9.8±2.7)	238.2±7.1* (18.1±3.1)	246.9±14.3 (12.2±2.0)	259.2±11.5 (10.9±4.0)	278.3±6.2 (4.3±3.1)
CFDH (VI D)	50	285.2±4.8	271.2±5.2 (4.9±0.6)	251.6±6.0 (11.7±2.1)	230.6±7.5* (19.1±2.7)	249.8±8.1 (11.3±2.8)	254±10.1 (10.9±3.3)	276.5±4.4 (3.0±2.2)
EAFDH (VII D)	50	287.6±8.1	275.6±8.2 (4.1±0.3)	262.4±11.0 (8.7±2.9)	237.5±8.9* (17.3±3.5)	223.4±13.5* (22.3±4.6)	256.4±4.2 (10.3±2.9)	278.7±6.1 (3.0±2.8)
EAFDH (VIII D)	100	286.9±6.8	274.1±2.9 (4.4±2.2)	258.5±4.9 (9.8±2.9)	235.9±6.0* (17.7±3.7)	221±9.8* (22.3±4.8)	259.9±4.4 (9.3±2.4)	277.3±2.9 (3.2±2.6)
AQFDH (IX D)	75	288.3±3.9	278.4±2.7 (3.4±1.1)	253.4±4.4 (12.0±1.9)	223.5±3.6* (21.5±1.2)	231.7±6.0* (19.7±1.9)	262.8±4.1 (8.8±1.5)	275.1±4.4 (4.5±0.8)
AQFDH (X D)	150	278.0±14.3	266.6±11.0 (4.0±1.1)	249.0±6.6 (10.2±3.2)	228.5±5.4* (17.6±4.6)	216.9±7.7** (21.8±5.8)	251.7±9.1 (9.2±5.6)	272.8±4.1 (1.6±5.0)

PEFDH: Petroleum ether fraction of DH, CFDH: Chloroform fraction of DH, EAFDH: Ethyl acetate fraction of DH, AQFDH: Aqueous fraction of DH, DH: *Decalepis hamiltonii*

Table 2: Effect of fractions of ST on blood glucose levels in alloxan-induced diabetic rats

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours after the treatment						
		0 hr	2 hr	4 hr	6 hr	8 hr	16 hr	24 hr
Normal (I)	-	88.5±5.9	87.2±6.05 (1.4±0.4)	84.7±7.3 (4.3±0.5)	82.3±6.3 (7.02±1.5)	83.01±4.6 (6.1±1.7)	83.4±4.7 (5.6±1.5)	82.5±5 (6.7±0.1)
Diabetic control (II)	-	281.9±9.7	276.8±10.3 (1.7±1.2)	268.1±10.4 (4.7±3.2)	264.8±22.5 (6.1±3.7)	265.2±17.8 (5.9±4.2)	268.0±17.1 (5.1±3.7)	271.1±9.41 (3.9±2.5)
Glibenclamide (III)	5	265.95±11.9	217.69±14.0 (18.0±5.5)	175.79±18.6 (33.9±6.8)	189.3±17.1 (28.7±6.5)	227.9±19.73 (14.28±6.5)	242±14.7 (9.0±4.6)	257±9.2 (3.3±2.1)
PEFST (III E)	140	275.8±18.6	244.4±11.1 (11.1±5.0)	249.1±10.2 (10.5±2.3)	244.4±6.2 (11.2±2.3)	257.1±5.3 (6.4±3.1)	266.4±3.4 (3.0±6.1)	275.8±2.5 (−0.4±0.1)
PEFST (IV E)	280	274.9±14.5	259.8±10.5 (9.6±3.2)	253.15±10.2 (10.2±5.9)	249.6±4.8 (15.3±5.1)	254.2±5.2 (11.3±5.3)	267.4±3.1 (2.4±1.8)	279.7±5.1 (−2.0±0.5)
CFST (V E)	50	288.7±11.3	243.9±13.1 (15.4±5.6)	240.1±11.3 (17.1±4.0)	235.4±3.6* (18.1±2.6)	249.9±3.8 (19.3±3.4)	261.6±4.8 (9.3±2.6)	275.7±3.3 (4.4±3.2)
CFST (VI E)	100	283.2±15.7	238.7±8.9 (15.5±4.1)	249.7±13.2 (12.4±4.3)	256.8±8.0 (13.8±3.3)	266.9±6.3 (16.1±3.9)	265.1±5.7 (9.7±5.1)	275.8±3.4 (2.3±1.9)
EAFST (VII E)	100	286.0±6.7	258.2±3.8 (9.7±1.3)	227.7±15.5* (20.3±6.1)	223.1±5.3* (24.9±2.4)	258.1±5.1 (9.7±2.6)	268.2±4.0 (6.1±2.9)	278.3±4.6 (2.6±1.8)
EAFST (VIII E)	200	286.7±9.3	258.5±3.8 (9.7±1.8)	217.5±9.7** (24.1±2.6)	233.4±8.2* (18.6±2.1)	248.7±8.6 (13.2±2.1)	262.9±2.8 (8.2±2.3)	276.9±3.8 (3.3±2.0)
AQFST (IX E)	100	282.5±12.3	250.4±4.2 (11.2±2.1)	233.0±5.4* (17.3±5.0)	221.6±2.7* (19.5±3.4)	229.8±4.2* (18.5±4.5)	259.7±4.2 (7.9±1.2)	277.02±4.0 (1.8±0.6)
AQFST (X E)	200	280.5±17.0	254.0±12.0 (9.3±2.9)	231.8±5.2* (17.1±3.6)	220.1±10.4* (21.9±4.3)	225.7±5.7* (19.5±4.4)	248.1±7.2 (11.3±1.9)	272.7±1.7 (2.4±0.1)

n=6, *p<0.05, **p<0.01, Compared to the diabetic control at the respective time point; values given in parenthesis are percent blood glucose reduction. PEFST: Petroleum ether fraction of ST, CFST: Chloroform fraction of ST, EAFST: Ethyl acetate fraction of ST, AQFST; Aqueous fraction of ST, ST: *Shorea tumbuggaia*

subsequently 1 ml TBA (0.67%w/v) was added and all the tubes were kept in a boiling water bath for 20 minutes. The tubes were shifted to ice bath and centrifuged at 2500 ×*g* for 10 minutes. The amount of malonldialdehyde (MDA) formed in each of samples was assessed by measuring the optical density of the supernatant at 535 nm allied with reagent blank without tissue homogenate. The molar extinction coefficient for MDA was taken to be 1.56×10^5/M/cm.

Calculation:
μM/mg tissue = 3×absorbance of sample/50.156× (mg of tissue taken)

Estimation of GSH from liver PMS

Glutathione was assayed by the method [16]. An aliquot of 1 ml of hepatic PMS (10%w/v) was mixed with 1 ml of sulphosalicylic acid (4%w/v) and centrifuged at 1200 g for 5 minutes and filtered. From the above, 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1M, pH 7.4), and 0.2 ml DTNB (40 mg/10 ml of phosphate buffer 0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow color developed was comprehended at 412 nm on a spectrophotometer.

Estimation of SOD from liver PMS

Super oxide dismutase activity was estimated method [14]. The reaction mixture consisted of 0.5 ml of hepatic PMS, 1 ml 50 mM sodium carbonate, 0.4 ml of 25 μM nitro blue tetrazolium (NBT), and 0.2 ml, 0.1 mM EDTA. The reaction was initiated by addition of 0.4 ml of 1 mM hydroxylamine-hydrochloride. The change in absorbance was recorded at 560 nm. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required inhibiting the reduction of NBT by 50%.

Estimation of CAT from liver PMS

CAT activity was assayed [9]. The assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7), 1 ml H_2O_2 (0.019 M), 0.05 ml of hepatic PMS (10%w/v). Changes in absorbance were recorded at 240 nm for 2 minutes with 60 seconds interval using a spectrophotometer (Model 106).

Statistical analysis

Data for various parameters were analyzed using analysis of variance and the group means were compared by Tukey-Kramer test (Graph Pad Version 3.06, La Jolla, CA, USA). Values were considered statistically significant when at $p<0.05$.

RESULTS

Effect of DH and ST fractions on blood glucose levels in normoglycemic rats (OGTT)

In OGTT, DH and ST significantly reduced the blood glucose levels in glucose loaded rats at 30 minutes and 60 minutes. A significant ($p<0.05$) decrease in the blood glucose level of treated glucose loaded rats as compared with control rats was observed. The EAFDH, AQFDH and EAFST, AQFST were possessed significant effect in 30 minutes and 60 minutes at the doses of 200 mg/kg and 400 mg/kg. The effect was similar to glibenclamide.

EDH and ST fractions on body weight in alloxan-induced diabetic rats

Animals treated with alloxan in diabetic control group showed increase in body weight, which was persistently observed till the end of the study period i.e., 28 days. Where as in fractions of DH treated groups the body weight was restored almost it's near initial values after 28 days of treatment.

EDH and ST fractions on blood glucose levels in alloxan-induced diabetic rats

A statistically significant ($p<0.001$) decrease was observed in the blood glucose levels of diabetic rats treated with fractions of DH at the dose of 200 mg/kg when compared with diabetic control rats from the 7th day experimental period. Glibenclamide causes maximum reduction in blood glucose levels from 1st week on wards when compared to controlled diabetic rats.

EDH and ST fractions on insulin and HbA1c levels in alloxan-induced diabetic rats

Significant change ($p<0.05$) was noted in the serum insulin levels and HbA1c levels of the diabetic animals treated with fractions of DH, there by suggesting that DH probably exerts antihyperglycemic activity by a pancreatic mechanism dependent of insulin secretion (Table 3).

EDH and ST fractions on various lipid parameters in alloxan-induced diabetic rats

The marked hyperlipidemic that characterizes the diabetic state may, therefore, be regarded as a consequences of the uninhibited actions of lipolytic hormones on the fat depots. Administration of EDH at both 200 mg/kg and 400 mg/kg doses caused reduction of TC, TG, LDL, VLDL, and improved HDL level significantly ($p<0.001$).

EDH and ST fractions on DPPH, reducing power, hydroxyl radical scavenging activity in alloxan-induced diabetic rats

The effect of alcoholic fractions of DH on inhibition of hydroxyl radical reduction was assessed by iron (II)-dependent deoxyribose damage assay. The capability of alcoholic fractions of DH of reducing hydroxyl radical production at all concentration was shown in Table 6. Ascorbic acid, used as standard was highly effective in inhibiting the oxidative DNA damage, showing an IC_{50}=18.46 μg/ml. Where as the plant product have IC_{50}=35.56 μg/ml, which can be comparable to standard. The dose dependent inhibition of DPPH radical indicated that a fraction of DH and ST causes reduction of DPPH radical in stoichiometric manner. The

Table 3: Effect of petroleum ether, chloroform, ethyl acetate, and aqueous fractions of DH and ST on oral glucose tolerance in normal rats

Groups	Dose (mg/kg)	Blood glucose levels (mg/dl)				
		0 minute	30 minutes	60 minutes	90 minutes	120 minutes
Normal control		87.5±3.5	154.6±28.4	159.6±7.8	125.6±9.7	118.4±11.8
Glibenclamide	5	86.6±3.6	126.5±19.5	107.6±10.5*	95.6±11.4*	75.4±3.6**
PEFDH	60	87.34±2.4	124.56±13.4	112.34±12.3	109.3±11.3	100.3±2.3*
CFDH	25	88.67±3.9	132.4±12.4	125.7±13.2	119.8±12.3	108.9±3.8
EAFDH	50	89.5±5.7	154.9±21.4	115.8±12.4**	100.3±11.9**	78.6±5.7**
AQFDH	75	85.8±2.8	189.7±29.5*	100.5±21.6**	84.6±13.4**	72.3±5.6**
PEFST	140	86.3±2.7	152.3±21.3	148.56±18.7	121.3±11.3	108.8±4.5
CFST	50	88.3±3.6	157.8±18.5	138.9±14.5	120.5±2.8	107.8±5.6
EAFST	100	85.4±3.7	178.4±22.4*	125.7±10.6 *	102.5±12.4*	82.3±13.4*
AQFST	100	84.5±3.5	178.5±27.6*	132.5±9.8*	93.5±7.8**	71.5±5.6**

n=6; *p<0.05, **p<0.001, compared to diabetic control at the respective time point, PEFDH: Petroleum ether fraction of DH, CFDH: Chloroform fraction of DH, EAFDH: Ethyl acetate fraction of DH, AQFDH: Aqueous fraction of DH, PEFST: Petroleum ether fraction of DH, CFST: Chloroform fraction of DH, EAFST: Ethyl acetate fraction of ST, AQFST: Aqueous fraction of ST, DH: *Decalepis hamiltonii*, ST: *Shorea tumbuggaia*

Table 4: Effect of PEFDH, CFDH, EAFDH, PEFST, CFST, EAFST, AQFDH, and AQFST on different parameters in alloxan-induced type 2 diabetic rats

Groups	Dose (mg/kg)	Body weight in g					Blood glucose (mg/dl)					Serum TG (mg/dl)				
		1st day	After				1st day	After				1st day	After			
			7 days	14 days	21 days	28 days		7 days	14 days	21 days	28 days		7 days	14 days	21 days	28 days
Normal (I)		182.56±1.2	180.23±2.3	184.5±1.3	193.3±2.1	185.5±2.3	86.7±3.12	88.3±4.5	87.4±3	87.3±4.3	88.4±5.6	34.21±1.3	35.88±3.4	37.42±3.4	37.08±5.4	38.4±4.9
Diabetic control (II)	-	141±2.3	142±2.5	140.1±3.7	138.3±2.2	135±5.2	210.34±4.2	217.8±8.4	234.8±9.4	248.9±12.3	269.9±19.3	92.8±4.2	121.4±10.7	124.99±12.4	138.09±14.2	142.37±14.2
Glibenclamide (III)	5	131.5±3.5	140.4±4.1*	145±3.2*	151.3±3.5*	155.8±4.1*	210.9±15.5	165.03±14.5**	109.83±4.47**	94.9±15.4**	84.9±16.8**	84.5±2.3	60.9±4.4**	52.3±4.3**	50.4±3.2**	45.8±4.2**
PEFDH (IV)	60	142.3±3.2	143.2±3.6	147.8±3.9	143.5±3.2	146.8±4.2	279.5±6.9	234.5±5.6	215.6±4.6	201.3±4.7	198.7±5.3	95.4±2.5	99.5±5.4	102.3±2.4	109.4±3.8	121.3±4.6
CFDH (V)	25	144.2±2.6	145.7±3.2	143.5±3.8	146.8±2.7	145.7±3.8	278.3±6.2	256.4±4.7	248.9±6.7	224.6±7.3	206.7±5.7	98.5±3.6	100.7±5.8	108.5±5.8	111.3±5.6	120.3±3.6
EAFDH (VI)	50	135.5±3.4	144.1±3.8*	139.5±1.4**	148±3.5*	156.7±2.8*	223.4±7.9	194.45±12.38*	174.5±6.8*	102.6±32.3**	86.94±2.9**	94.3±1.4	85.2±9.8*	71.08±4.3*	53.4±3.2**	39.4±4.3**
AQFDH (VII)	75	156.7±2.7	165.4±3.4*	147.8±5.6*	168.5±4.7*	176.5±3.56*	223.4±7.9	201.45±12.3*	174.5±6.8*	125.6±23.3**	86.94±2.9**	96.8±5.4	87.9±2.3**	70.4±4.3**	58.4±5.6**	43.4±3.2**
PEFST (VIII)	140	148.5±5.4	151.2±5.4	148.4±4.7	153.4±3.8	134.6±4.3	229.6±5.7	213.4±12.7	201.4±6.9	206.8±3.6	198.7±6.8	98.6±6.8	95.4±3.6	89.6±4.6	79.7±5.8	89.6±4.8
CFST (IX)	50	153.4±6.7	148.9±5.7	149.0±5.9	143.2±4.8	153.6±7.9	232.5±5.9	212.4±11.3	208.6±5.9	205.7±5.9	199.5±3.7	100.4±4.6	97.6±4.7	89.5±2.7	93.5±4.8	96.8±4.7
EAFST (X)	100	133.6±4.1	145.3±2.3*	142.6±4.5*	147.6±3.8*	156.3±5.6*	256.4±5.8	176.45±4.9**	156.56±5.6*	123.5±20.4**	98.34±5.8**	85.9±3.9	94.3±5.3*	79.3±6.4*	54.2±3.4**	41.2±5.4**
AQFST (XI)	100	168.4±3.1	156.46±2.5*	125.4±2.4**	148.3±5.6*	167.3±4.5*	248.4±10.2	202.8*±10.8	142.4±11.84*	101.4±18.3*	79.4±9.9**	98.5±4.8	89.9±4.1**	73.43±3.2**	50.4±4.5**	39.4±5.6**

N=6; *p<0.05, **p<0.01, when compared to diabetic control at the respective time point; PEFDH: Petroleum ether fraction, CFDH: Chloroform fraction, EAFDH: Ethyl acetate fraction, AQFDH: Aqueous fraction of DH, EAFST: Ethyl acetate, PEFST: petroleum ether fraction, CFST: chloroform fraction, AQFST: Aqueous fraction of ST, DH: *Decalepis hamiltonii*, ST: *Shorea tumbuggaia*, TG: Triglycerides

Table 5: Effect of PEFDH, CFDH, EAFDH, PEFST, CFST, EAFST, AQFDH, and AQFST on different parameters in alloxan-induced type 2 diabetic rats

Groups/dose (mg/kg)	Serum cholesterol (mg/dl)					Serum HDL (mg/dl)					Serum LDL (mg/dl)				
	1st day	After				1st day	After				1st day	After			
		7 days	14 days	21 days	28 days		7 days	14 days	21 days	28 days		7 days	14 days	21 days	28 days
Normal (I)	53.21±2.1	56.41±4.32	56.12±5.9	54.21±6.6	55.21±4.4	34.24±2.6	36.52±2.3	33.07±6.4	34.84±4.41	35.43±5.5	9.23±2.1	11.76±2.4	10.12±2.4	11.42±3.1	12.43±4.32
Diabetic control (II)	184.5±12.2	207.96±12.5	255.72±14.3	268.4±15.6	272.46±13.9	36.46±4.5	29.19±1.34	16.24±1.02	13.91±2.2	10.46±2.33	129.48±2.9	156±16.8	214.36±32.4	226.59±20.9	232.92±22.1
Glibenclamide (III)	145.6±18.7	143.43±11.2*	92.46±3.34**	68.46±4.3**	54.22±4.5**	32.48±2.3	24.85±1.24*	35.42±2.43*	38.55±2.64**	41.38±3.4**	96.22±3.6	105.38±2.9**	45.96±3.1**	17.61±3.5**	3.04±2.01**
PEFDH (IV)	134.6±12.3	142.5±10.4	128.9±3.4	120.6±4.5	118.7±5.7	38.95±3.8	27.9±2.7	32.67±3.6	30.56±2.8	32.38±5.8	76.57±4.2	94.7±3.2	75.77±3.7	68.16±3.9	62.06±2.9
CFDH (V)	145.7±11.3	140.5±11.4	129.6±4.6	121.7±5.7	120.5±4.8	37.9±2.7	35.8±3.6	34.2±2.9	37.4±3.8	38.9±3.8	88.1±3.8	84.56±3.2	73.7±2.8	62.04±2.8	57.54±2.7
EAFDH (VI)	164.2±11.4	159.42±4.3	112.48±5.12*	83.4±5.9**	59.4±10.3**	29.24±5.9	26.15±2.34*	31.98±3.45*	37.54±4.45*	39.2±5.54*	126.1±5.1	26.15±2.34**	31.98±3.45*	37.54±4.45**	39.2±5.54**
AQFDH (VII)	186.9±4.3	55.43±3.47**	96.36±4.22**	73.18±5.09**	60.50±4.43**	21.42±5.2	26.78±1.96	34.5±2.43**	38.4±3.39**	39.9±4.48**	116.12±1.9	111.1±2.4**	47.78±2.09**	23.10±2.2**	11.92±1.67**
PEFST (VIII)	174.6±5.4	120.4±3.78	109.7±6.8	102.8±4.8	100.3±3.6	32.6±3.8	34.6±5.7	28.9±2.6	37.9±3.6	31.4±3.8	122.28±3.6	66.72±13.45	62.88±5.6	48.96±7.9	50.98±2.9
CFST (IX)	176.6±5.7	159.8±3.56	148.9±4.8	121.4±3.9	103.6±4.7	36.8±2.5	23.5±3.1	20.45±2.7	32.5±2.1	34.6±4.2	119.45±2.8	116.78±3.8	110.55±4.8	80.2±10.4	49.64±3.8
EAFST (X)	159.3±9.4	143.42±5.2	98.42±6.14**	73.4±9.8**	54.4±11.4**	34.56±6.2	25.18±4.5*	33.18±4.2*	39.54±5.34**	40.43±5.67**	122.88±2.6	25.18±4.5**	33.18±4.2**	39.54±5.34**	40.43±5.67**
AQFST (XI)	179.5±7.4	149.99±12.15	71.01±2.22**	62.29±4.43**	56.53±9.77**	24.56±8.2	28.45±1.084	35.4±2.66**	39.5±3.50**	40.9±2.94**	110.57±3.6	105.56±1.6**	20.93±16.23**	12.71±3.4**	7.75±2.3**

N=6; *p<0.05, **p<0.01, when compared to diabetic group at the respective time point. PEFDH: Petroleum ether fractions of DH, CFDH: Chloroform fraction of DH, EAFDH: Ethyl acetate fraction of DH, AQFDH: Aqueous fraction of DH, EAFST: Ethyl acetate fraction of DH, PEFST: Petroleum ether fraction of ST, CFST: Chloroform fraction of ST, AQFST: Aqueous fraction of ST, DH: *Decalepis hamiltonii*, ST: *Shorea tumbuggaia*, LDL: Low-density lipoproteins, HDL: High-density lipoproteins

Table 6: Effect of PEFDH, CFDH, EAFDH, EAFST, PEFST, CFST, AQFDH, and AQFST on different parameters in alloxan induced type 2 diabetic rats

Groups	Dose (mg/kg)	Serum VLDL (mg/dl)					Serum insulin levels (µIU/ML)	Serum HbA1c (g/dl)
		1st day	After					
			7 days	14 days	21 days	28 days		
Normal (I)		9.12±2.1	8.13±2.1	7.93±2.3	7.98±2.1	7.35±1.3	11.5±2.6	4.05±0.403
Diabetic control (II)		18.56±1.6	24.09±3.2	25.12±4.5	27.9±2.8	29.8±3.5	4.23±2.1	10.29±1.210
Glibenclamide (III)	5	16.9±1.4	13.2±5.43**	11.1±0.98**	12.3±1.34**	9.8±2.34**	9.7±2.43**	3.95±0.621**
PEFDH (IV)	60	19.08±2.4	19.9±2.7	20.46±1.9	21.88±1.9	24.26±2.1	4.03±2.4	8.34±1.4
CFDH (V)	25	19.7±2.5	20.14±2.9	22.26±3.6	22.26±2.6	24.06±2.9	5.89±3.5	7.89±2.7
EAFDH (VI)	50	18.86±2.3	17.3±4.23	14.5±0.98**	10.9±0.98**	7.9±0.98**	9.6±0.76**	4.3±1.9**
AQFDH (VII)	75	19.36±2.7	17.54±1.3*	14.08±1.3**	11.68±1.3**	8.68±2.1**	9.90±2.5**	4.69±4.5**
PEFST (VIII)	140	19.72±2.9	19.08±1.5	17.92±3.1	15.94±3.7*	17.92±3.6	4.21±2.8	6.45±2.6
CFST (IX)	50	20.08±1.7	19.52±1.6	17.9±1.6	18.7±3.2	19.36±1.3	5.6±2.3	8.4±2.67
EAFST (X)	100	17.18±1.8	19.1±0.23	15.9±2.3**	10.9±0.83**	8.23±0.9**	9.09±0.548**	4.6±1.03**
AQFST (XI)	100	19.36±2.9	15.98±2.1**	14.68±1.56**	10.08±2.1**	7.88±1.34**	9.02±4.5**	4.08±3.9**

N=6; *p<0.05, **p<0.01, when compared to diabetic group at the respective time point; PEFDH: Petroleum ether fraction of DH, CFDH: Chloroform fraction of DH, EAFDH: Ethyl acetate fraction of DH, AQFDH: Aqueous fraction of DH, PEFST: Petroleum ether fraction of ST, CFST: Chloroform fraction of ST, EAFST: Ethyl acetate fraction of ST, AQFST: Aqueous fractions of ST, DH: *Decalepis hamiltonii*, ST: *Shorea tumbuggaia*, VLDL: Very low density lipoproteins, HbA1c: Glycosylated hemoglobin A1c

Table 7: Effect of PEFDH, CFDH, EAFDH, EAFST, PEFST, CFST, AQFDH, and AQFST on different *in-vivo* antioxidant parameters in alloxan-induced type 2 diabetic rats

Group	Dose (mg/kg)	CAT (µM/mg tissue)	GSH (µM/mg tissue)	LPO (µM/mg tissue)	SOD (U/mg tissue)
Normal (I)		0.0576±0.00168	0.185±0.0033	0.0492±0.00062	0.03174±0.0077
Diabetic control (II)		0.0043±0.00014[a]	0.0405±0.00144[a]	0.476±0.00522[a]	0.00432±0.0037[a]
Glibenclamide (III)	5	0.0666±0.00186[ab]	0.179±0.00393[ab]	0.0582±0.0004[ab]	0.0258±0.0043[ab]
PEFDH (IV)	60	0.0057±0.0013[a]	0.0567±0.0014[a]	0.3671±0.00278[a]	0.00789±0.00234[a]
CFDH (V)	25	0.0078±0.0034[a]	0.0732±0.0012[a]	0.432±0.00156[a]	0.00687±0.00345[a]
EAFDH (VI)	50	0.0632±0.0032[ab]	0.0683±0.0012[ab]	0.04324±0.0043[ab]	0.01923±0.2134[ab]
AQFDH (VII)	75	0.0456±0.00123[ab]	0.145±0.0056[ab]	0.0345±0.0025[ab]	0.01987±0.0035[ab]
PEFST (VIII)	140	0.0078±0.0013[a]	0.0489±0.00156[a]	0.325±0.00367[a]	0.00694±0.00342[a]
CFST (IX)	50	0.0049±0.00125[a]	0.0589±0.0025[a]	0.215±0.0034[a]	0.0034±0.00189[a]
EAFST (X)	100	0.0598±0.00148[ab]	0.0678±0.00178[ab]	0.02953±0.0017[ab]	0.01724±0.081[ab]
AQFST (XI)	100	0.0563±0.00145[ab]	0.201±0.0067[ab]	0.0510±0.0034[ab]	0.02015±0.0045[ab]

N=4, [a]p<0.001, compared to normal rats. [b]p<0.001, compared to diabetic control animals. PEFDH: Petroleum ether fraction of DH, CFDH: Chloroform fraction of DH, EAFDH: Ethyl acetate fraction of DH, AQFDH: Aqueous fraction of DH, EAFST: Ethyl acetate fraction of ST, PEFST: Petroleum ether fraction of ST, CFST: Chloroform fraction of ST, AQFST: Aqueous fraction of ST, LPO: Lipid-peroxidation, SOD: Superoxide dismutase, CAT: Catalase, GSH: Reduced glutathione, DH: *Decalepis hamiltonii*, ST: *Shorea tumbuggaia*

Table 8: Effect of PEFDH, CFDH, EAFDH, EAFST, PEFST, CFST, AQFDH, and AQFST on different *in-vitro* antioxidant parameters by DPPH scavenging activity

Concentration	Percentage of inhibition DPPH peak area								
	Ascorbic acid	PEFDH	CFDH	EAFDH	AQFDH	PEFST	CFST	EAFST	AQFST
5	18.34±0.340	12.34±0.02	8.9±0.03	12.45±0.04	7.62±0.2	5.6±0.3	4.8±0.03	15.60±0.010	2.32±0.1
10	26.87±0.560	23.45±0.01	16.7±0.07	21.40±0.08	18.07±0.5	14.3±0.02	7.9±0.2	18.56±0.990	9.6±0.4
20	34.56±0.230	38.9±0.05	23.5±0.08	35.60±0.09	27.89±0.8	21.3±0.03	18.9±0.4	23.40±0.020	14.67±0.5
40	56.78±0.060	48.9±0.04	28.9±0.09	48.90±0.12	34.63±1.7	27.8±0.5	26.8±0.6	34.50±0.050	20.9±0.7
60	78.90±0.080	69.8±0.12	32.5±0.03	67.80±0.11	59.18±0.5	34.6±0.7	39.0±0.7	48.67±0.060	34.67±0.5
80	87.60±0.090	84.6±0.02	45.6±0.08	78.90±0.21	73.56±0.02	45.6±0.8	45.7±0.3	65.40±0.080	46.1±0.8
100	100.00±0.088	94.5±0.03	59.02±0.05	123.40±0.22	99.42±50.02	53.6±0.2	57.9±0.6	79.50±0.045	61.2±0.2
IC_{50} (µg/ml)	25.20	47.8	102.9	29.86	42.14	97.9	125.8	50.70	84.79

The values are mean±SEM; the values are taken in triplicate form. PEFDH: Petroleum ether fraction of DH, CFDH: Chloroform fraction of DH, EAFDH: Ethyl acetate fraction of DH, AQFDH: Aqueous fraction of DH, EAFST: Ethyl acetate fraction of ST, PEFST: Petroleum ether fraction of ST, CFST: Chloroform fraction of ST, AQFST: Aqueous fraction of ST, DPPH: 2,2-diphenyl-1-picrylhydrazyl, SEM: Standard error of the mean, DH: *Decalepis hamiltonii*, ST: *Shorea tumbuggaia*

IC_{50} values of fractions of DH and are found to be 29.86 µg/ml, which can be comparable to ascorbic acid (IC_{50}=25.20 µg/ml) as a standard compound. In reducing power method the increase in concentration causes increase in absorbance. This reduction could have resulted from the antioxidant effect of the different concentrations of fraction of plant DH and ST, whose phytochemical components include flavonoid, which is known for antioxidant effect. EDH and ST fractions on liver TBAR, SOD, GSH, and CAT enzyme activity in alloxan-induced diabetic rats diabetic subjects have been shown to have increased oxidative stress and decreased antioxidant level.

DISCUSSION

The present study was undertaken with the objective of exploring the antidiabetic potential of DH and ST, in alloxan-induced diabetic rats, through blood glucose levels, lipid metabolism, and antioxidant status.

No toxic reactions were observed thereby suggesting the non-toxic nature of DH and ST at the selected doses till the end of the experimental period. Alloxan causes diabetes by the rapid depletion of β-cell and thereby brings about a reduction in insulin release. As well as alloxan produces oxygen radicals in the body, this causes pancreatic injury and role in the alleviation of diabetes. Hyperglycemia causes oxidative damage by the generation of reactive oxygen species [32] and results in the development of diabetic complications. Decreased antioxidant enzyme levels and enhanced LPO have been well-documented in alloxan-induced diabetes. In our study, administration of alloxan increased serum glucose levels when similar effect of alloxan was also observed in previous studies on the evaluation of Persea Americana [3] for antidiabetic activity. In OGTT, at 60, 90, and 120 minutes, a significant decrease in the blood glucose levels was observed in treated rats as compared with control rats. From the OGTT data, it is clear that administration of PEFDH, CFDH, EAFDH, and AQFDH of AQFST on living β-cells of islets of langerhans to release more insulin. A number of other plants have been observed to exert antidiabetic activity through insulin-release stimulatory effects, like *Musasapientum* [23]. HbA1c was found to increase in patients with diabetes mellitus to about 16% [20] and the amount of increase is directly proportional to the fasting blood glucose levels [18]. There is evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition [24]. In the present study, the diabetic rats had shown higher levels of HbA1c compared to those in normal rats. Treatment with various fractions of DH and ST glibenclamide showed a significant decrease in HbA1c levels in diabetic rats that could be due to an improvement in glycemic status. Under normal circumstances, insulin activates enzyme lipoprotein lipase causes hydrolysis TG [23]. In diabetic rats, an increase in TC, TG, LDL and VLDL-cholesterol, decrease in HDL-cholesterol. The abnormal high concentration of serum lipids in diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase [26] proved insulin concentration significantly, when treated with different fractions of these plants indicates the insulin secretagogues activity as well as antihyperlipidemic activity. The effect of alcoholic fractions of DH and ST on inhibition of hydroxyl radical production was assessed by iron (II)-dependent deoxyribose damage assay. Ferrous salts can react with hydrogen peroxide thus, forming hydroxyl-radical via Fenton's reaction. The iron required for this reaction is obtained either from the pool of iron or the heme-containing proteins. The hydroxyl radical thus, produced may attack the sugar of DNA bases, which tends to sugar fragmentation; base loss and DNA strand breakage. Addition of transition metal ions like iron at low concentration to deoxyribose causes degradation of the sugar into malondialdehyde and other related compounds which form a chromogen with TBA. The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto-oxidation [7]. The radical scavenging activity of fractions of DH was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH radical. Lipid peroxide was found to be significantly high in the diabetic group as compared to the normal group, which increases TBARS activity. This may be due to the presence of phenols and flavonoids, which may have a major role in reducing oxidative stress associated with diabetes [25]. Apart from lipid peroxides, CAT, super oxide dismutase, glutathione synthetase are examples of enzymatic antioxidants. SOD and CAT are considered as primary enzymes, since they are involved in the direct elimination of reactive oxygen species. SOD is an important defense enzyme which catalyzes the dismutase of superoxide radicals and CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive OH radicals. Glutathione synthetase, the most important biomolecule protecting against chemical-induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxide in the presence of glutathione peroxides (Meister *et al.,* 1984). The decreased level of glutathione synthetase observed in diabetic animals represents an increased utilization resulting from oxidative stress [5]. Restoring all the enzymatic and nonenzymatic antioxidant parameters in liver is similar to previously reported plants by [24].

CONCLUSION

The results of the present investigation clearly indicate that the fractions of DH and ST have glucose lowering effect on alloxan-induced diabetic rats. It was also found to be highly effective in managing the complications associated with diabetes mellitus, such as body weight maintenance, hyperlipidemia, and as an antioxidant prevents the defects in lipid metabolism. Therefore, DH and ST show therapeutic promise as a protective agent against the development of a major complication due to free radicals. This could be useful for prevention or early treatment of diabetic disorder. Further studies are in progress to isolate, identify, and characterize the active principles.

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