

Anti-Diabetic Assessment of the Hydro-Alcoholic Leaf Extracts of the Plant *Tetrastigma angustifolia* (Roxb.) a traditionally used North-Eastern Indian vegetable

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycaemia generally associated with oxidative stress. The present study aims to evaluate the hypoglycaemic activity of hydro-ethanolic (3:7) leaf extracts of *Tetrastigma angustifolia* (Roxb.) (Family: Vitaceae) to streptozotocin induced diabetic wistar albino rats. The blood glucose level was measured using single touch blood glucose test strips and glucometer on weekly intervals until the end of 21st day. Other blood parameters like total lipid and liver profiles were investigated after oral administration of extracts to diabetic rats after the treatment of three weeks. The Histopathological changes of the diabetic and vehicle control rats were also investigated in this study. Daily oral administration of hydro-alcoholic leaf extracts (HSE 250 mg/kg & 500 mg/kg body weight) and metformin hydrochloride (5 mg/kg body weight) showed improvement effect on blood glucose level as well as hyperlipidaemia and liver functions due to streptozotocin induced diabetes (Type II). The extract has beneficial effects to maintain the animal body weight and has the regenerating power to pancreatic cells. The hydro-alcoholic leaf extracts dose fractions of *Tetrastigma angustifolia* (Roxb.) exhibited significant anti-hyperglycemic activities. The extract showed improvement in parameters like body weight and lipid profiles as well as regeneration of β -cells of pancreas and so might be of value for the treatment of diabetes (Type- II).

Key words: Anti-diabetic, glucose tolerance test, hypoglycaemia, *Tetrastigma angustifolia*.

INTRODUCTION

Diabetes mellitus (DM), both insulin-dependent DM (IDDM) and non-insulin dependent DM (NIDDM) is a common and serious metabolic disorder affecting the citizens of both developed and developing countries. Insulin is a protein hormone secreted by the β cells of islets of langerhans of pancreas. Deficiency of effective insulin in the body causes diabetes mellitus [1]. World Health organization (WHO) has predicted that the major burden will occur in developing countries. India has today become the diabetic capital of the world. Studies conducted in India in the last decade have highlighted that not only is

the prevalence of diabetes high but also that, it is increasing rapidly in the urban populations. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by 2025 [2]. The International Diabetes Federation and also the World Health Organization estimate that, worldwide over 100 million people suffer from type II diabetes and 50% of those cases are undiagnosed.

Medicinal herbs as potential source of therapeutics aids have attained a significant role in health system all over the world. From ancient times, plants have been catering as rich source of effective and safe medicines. About 80 % of world

populations are still dependent on traditional medicines.

The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments [3]. In India, around 15000 medicinal plants have been recorded [4] however traditional communities are using only 7,000 - 7,500 plants for curing different diseases [5-6].

Since the discovery of insulin several synthetic oral hypoglycemic drugs (OHDs) are available in market. However, these synthetic drugs are not sufficient to treat this deadly disease, particularly type II. Most of these synthetic drugs have some serious side effects. In this respect our traditional herbal therapeutic system can act as an alternative to synthetic drugs. Generally these phytochemicals have fewer side effects. Many of them are helpful in preventing the secondary complications of diabetes [7]. The herbal medicines are generally cost effective and readily accessible in compare to synthetic drugs. The adverse side effects and higher cost of the existing anti-diabetic drugs necessitate the search for novel, well tolerated, efficient and easily available herbal remedies to tackle the deadly diabetes. The plant *Tetrastigma angustifolia* is called as Naltanga in local language of North-East India. It is a member of family Vitaceae. In North – Eastern part of India, particularly Assam, the whole plant is used as a green vegetable and traditionally used to control diabetes. Literature survey revealed no scientific report on this plant grown in India with regards to anti-diabetic activity. The present work was undertaken to explore the anti-diabetic potential of hydro-alcoholic leaf extract (HSE) of *Tetrastigma angustifolia* in type 2 diabetic animals.

MATERIAL AND METHODS

Plant material

The plant *Tetrastigma angustifolia* was collected from the local market. The specimen was authenticated by Botanical Survey of India, Shillong and a voucher specimen (BSI/ERC/Tech./Plant Iden./2014/830) has been kept in our departmental library for future references. After authentication, fresh leaves were collected in bulk, shade dried

and pulverized in a mechanical grinder to obtain coarse powder.

Preparation of extract

The powdered leaf was extracted with ethanol and water (7:3) by cold maceration process. The extract was then filtered and evaporated to dryness at - 40 °C under reduce pressure in a lyophilizer. The dark brown mass was stored in a desiccator for future uses.

Drugs and Chemicals

Streptozotocin was procured from Sigma Aldrich Ltd. SGOT, SGPT, TC and ALP kits were purchased from SPAN Diagnostics Ltd. All standard chemicals were used in the present study and were prepared freshly as required.

Animal care and maintenance

Experiments were performed with 6-8 weeks old, healthy, male albino wistar rats, of body weight between 150-250 gm. Rats were housed under standard environmental conditions (25 ± 2° C temperature, 50 ± 5% humidity with a 12:12 hour dark and light cycle) and maintained with free access of water and a standard pellet (Lipton India. Ltd.). The animals were used with the approval of the Institute Animal Ethics Committee (Approval no: IAEC/DU/50 Dt. 24.9.13, Regd. No. 1576/Go/a/11/ CPCSEA dated 17.02.2012). Animals described as fasted were deprived of food for 12 hours but had free access to water.

Experimental Design of Anti-diabetic Activity:

Carboxy Methyl Cellulose (CMC) solution (0.3% w/v) was used as vehicle. The drugs were administered to animals orally using an intragastric tube daily for three weeks. Overnight fasted normal animals were randomly divided into five groups of six rats in each group.

Group I: Rats served as normal-control, which received vehicle i.e. 0.3% w/v of CMC solution.

Group II: Diabetic control which received Streptozotocin (55mg/kg body weight).

Group III: Diabetic rats received metformin hydrochloride 5 mg/kg body weight orally as standard drug.

Group IV: Rats were administered HSE of *T. angustifolia* 250 mg/kg- body weight with vehicle.

Group V: Rats were treated orally with HSE at the doses of 500 mg/kg body weight with vehicle.

The fasting blood glucose (FBG) was determined with the help of Gluco- meter and strips [8].

Acute Toxicity Studies

An acute toxicity study was carried out for the HSE Extract was dissolved in vehicle solution. Graded doses of 250 mg/kg & 500 mg/kg body weight were chosen based on acute toxicity studies done in wistar albino rat using OECD guidelines [9]. The HSE extract was administered orally. The animals were observed individually at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for total of 14 days.

Oral hypoglycemic effect of extracts in normal rats

The oral glucose tolerance test performed on overnight fasted normal animals. Rats divided

into four groups, were administered 0.3% CMC solution, metformin hydrochloride 5mg/kg, *T. angustifolia* leaf extract (250 mg/kg & 500 mg/kg) dissolved in vehicle respectively. Glucose (3g/kg-bw) was feed 30 minutes after the administration of extract. Blood was withdrawn from puncture of tail vein at 0, 30, 60, 90 and 120 min. of sample administration. Blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips [10].

Assessment of anti-hyperglycaemic activity in diabetic rats

Diabetes induced by injecting Streptozotocin intraperitoneally to overnight fasted albino rats. Streptozotocin weighted individually for each animal according to their weight and then solubilized with 0.2 ml normal saline just prior to administration. Diabetes was confirmed in streptozotocin injected rats by measuring the fasting blood glucose concentration, 72 hour after the injection. Rats with blood glucose level above 250

Table. 1: Report for the oral glucose tolerance test (OGTT) (mg/dl)

Animal Group	0 min	30 min	60 min	90 min	120 min
G-I Vehicle Control	78.16 ±3.22	140.50 ±3.12 ^{##}	152.16 ±2.40 ^{##}	167.00 ±2.72 ^{##}	186.51±3.20 ^{##}
G-II HSE(250 mg/kg)	76.32 ±3.16	135.12 ±1.85	155.32±2.13 ^{##}	124.12 ±2.13 ^{** ##}	117.35 ±2.44 ^{**##}
G-III HSE (500 mg/kg)	72.12 ±4.12	132.25 ±3.43	148.30±3.55 ^{##}	108.23±3.23 ^{**}	89.24 ±3.20 ^{**}
G-IV Standard drug (Metformin HCl)	80.15 ±1.51	128.15 ±3.21 ^{**}	118.20 ±1.86 ^{**}	101.32 ±1.62 ^{**}	86.21 ±1.53 ^{**}

Values are mean± SEM (n=6), statistical significance: *P<0.05, **P<0.01, compared with Normal control group I; #P<0.05 ##P<0.01, compared with Standard drug group IV.

Table. 2: The deviation of body weight of the animals during the treatment of hydro-alcoholic leaf extracts of *Tetragium angustifolia* during 21 days of treatment

Treatment	0 day (gm)	7 th day (gm)	14 th day (gm)	21 st day (gm)
Vehicle control(GROUP-I)	230.83±0.91	221.13±2.56 ^{ns}	238.33 ± 0.39 ^{##}	247.16 ± 0.69 ^{##}
Diabetic control (50 mg/kg STZ) (GROUP- II)	222.50±0.54	225.19±1.20 ^{ns##}	193.16±1.19 ^{**##}	183.66±0.89 ^{**}
Standard Drug(Metformin HCl 5mg/kg) (GROUP-III)	227.50± 0.73	210.24±1.32 ^{**##}	228.66±0.63 ^{**##}	232.83±0.55 ^{**##}
HSE (250 mg/kg)(GROUP-IV)	229.12± 2.32	233.15 ±1.25 ^{**##}	232.26± 2.21 ^{ns##}	237.12± 3.20 ^{**##}
HSE(500 mg/kg)(GROUP-V)	231.45 ± 2.15	235.18± 3.21 ^{**##}	233.2± 2.12 ^{ns##}	239.10± 2.80 ^{**##}

Values are mean± SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with Normal control group I; #P<0.05 ##P<0.01 compared with Diabetic control group II & ns – non significant.

mg/dl were considered diabetic and were used in this study. The rats were fasted overnight and blood was withdrawn by rupturing the tail vein. Blood samples were then collected for determination of blood glucose level on 0, 7, 14, and 21st day.

Biochemical Estimation

After 21st day of treatment, animals were sacrificed by cervical dislocation. Blood collected by cardiac puncture and serum was separated for determination of serum triglycerides (TG), high-density lipid cholesterol (HDL), low-density lipid cholesterol (LDL) and total cholesterol (TC) using commercial available kits (SPAN Diagnostics kit). The Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and lipid peroxidase, catalase and glutathione activity also measured for the assessment of anti-diabetic activity.

Histopathological Analysis

At the end of 21st day of treatment, the rats were sacrificed and the pancreas were removed and fixed for 4 days in 4 % formalin solution. Transverse sections of pancreases were obtained after making paraffin blocks and sectioning by rotary microtome and observed under photomicroscope for histopathological changes [11].

RESULTS

The acute toxicity studies revealed the non-toxic nature of the HSE. No toxic reactions were observed at any doses until the end of the experiment. The hydro-alcoholic extract showed a significant reduction in blood glucose levels from 90 min onwards (Table 1.) The deviation of body weight of experimental animals was observed at Table 2. In diabetic animals, significant reduction in the blood glucose level was noticed as compared

Table 3: The effect of HSE of *Tetragium angustifolia* on fasting blood glucose level on streptozotocin- induced diabetic rats.

Treatment	0day (mg/dl)	7 th day (mg/dl)	14 th day (mg/dl)	21 st day (mg/dl)
G-I- Vehicle control	92.53±1.87	103.36±3.31 [#]	101.12±4.29 [#]	98.15±2.23 [#]
G-II Diabetic control	255.23±3.35	278.41 ±4.32 ^{**}	282.15 ± 5.52 ^{**}	302.26 ±3.12 ^{**}
G-III Standard Drug	240.54±4.06	160.45±2.30 ^{**#}	135.13±6.40 ^{**#}	116.12±8.77 ^{**#}
G-IV HSE (250mg/kg)	246.12 ± 2.25	210.42 ± 3.30 ^{**#}	180.62 ± 4.48 ^{**#}	140.32 ± 3.38 ^{**#}
G-V HSE (500mg/kg)	241.42 ± 3.32	193.22 ± 5.30 ^{**#}	172.24	± 3.15 ^{**#}
128.43± 4.24 ^{**#}				

Values are mean± SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with Normal control group I; #P<0.05 ##P<0.01 compared with Diabetic control group II.

Table 4: Effects of HSE of *Tetragium angustifolia* on Cholesterol, Triglycerides, HDL, and LDL of control and experimental rats

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL(mg/dl)
G-I- Vehicle control	162.26 ±4.80 [#]	213.32±6.80 [#]	40 ±2.30 [#]	68.64 ± 3.80 [#]
G-II Diabetic control	230.05±6.50 ^{**}	302.12±4.90 ^{**}	25 ±2.20 ^{**}	136.43± 4.20 ^{**}
G-III Standard Drug	168.08±6.70 [#]	240.02±5.60 [#]	34 ±1.90 [#]	85.68 ± 3.80 [#]
G-IV HSE (250mg/kg)	198.25 ± 4.60 ^{**#}	263.45± 4.50 ^{**#}	33.12 ±1.50	102.47± 5.10 ^{**#}
G-V HSE (500mg/kg)	173.32 ± 6.50 [#]	232.30 ± 7.21	38.37± 3.20 [#]	82.23 ± 4.70 [#]

Values are mean± SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with Normal control group I; #P<0.05 ##P<0.01 compared with Diabetic control group II.

to the control (Table 3). Table 4 shows the effect of hydro- alcoholic leaf extracts on TC, LDL & on HDL. The results of biochemical parameters such as SGOT, SGPT and ALP presented in the Table 5. The level of lipid- peroxidase, glutathione and catalases were described in the Table 6. The histology of pancreas (Figure 1) showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control (a). In diabetic animals treated extensive damage to islets of langerhans and reduced dimensions of islets were observed in diabetic rats (b) which were restored toward normal cellular population size of islets by HSE 250 mg/kg and 500 mg/kg-bw treatment (c & d).

DISCUSSION

In our investigations, the oral glucose tolerance test and normoglycaemic studies revealed that the hydro-alcoholic extract of the leaf

has the capacity to lower blood glucose level. The Blood glucose level of control group reached a peak reduction at 30 minutes. Both doses of HSE (250 mg /kg & 500 mg/kg) decreased blood glucose level gradually at 90 and 120 minutes. Administration of metformin hydrochloride causes fall of glucose level continuously except at 30 minutes. The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues [12]. In this experiment, we used the streptozotocin for induction of diabetes to rats. Streptozotocin is a preferred agent to induce experimental diabetes since it has some advantages over alloxan such as, relatively longer half-life (15 mins), sustained hyperglycaemia for longer duration and the development of well-characterized diabetic complications with fewer incidences of ketosis as well as mortality [13]. Streptozotocin acts as a diabetic agent by the destruction of β -cells of pancreatic islets of

Table 5: Effect of HSE of *Tetragium angustifolia* on liver enzymes of control and experimental rats.

Groups	SGOT(U/L)	SGPT (U/L)	ALP (U/L)
G-I- Vehicle control	50.46 \pm 4.90 ^{##}	43.21 \pm 4.40 ^{##}	105 \pm 2.40 ^{##}
G-II Diabetic control	100.79 \pm 7.20 ^{**}	84.23 \pm 3.30 ^{**}	175 \pm 3.60 ^{**}
G-III Standard Drug	58.38 \pm 5.60 ^{###}	49.33 \pm 2.80 ^{###}	122 \pm 2.80 ^{**}
G-IV HSE (250mg/kg)	75.13 \pm 2.80 ^{###}	65.73 \pm 3.80 ^{##}	140 \pm 4.30 ^{###}
G-V HSE (500mg/kg)	61.23 \pm 4.50 ^{##}	52.62 \pm 4.50 ^{##}	120 \pm 3.40 ^{###}

Values are mean \pm SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with Normal control group I; #P<0.05 ###P<0.01 compared with Diabetic control group II.

Table 6. Effect of HSE of *Tetragium angustifolia* on liver enzymes of control and experimental rats

Groups	Lipid Peroxidase (nM MDA/mg of protein)	Glutathione (nM MDA/ mg of protein)	Catalase activity (iM of H ₂ O ₂ decomposed /min/ mg protein)
G-I-Vehicle control	15.057 \pm 0.83 ^{##}	52.699 \pm 0.56 ^{##}	13.04 \pm 0.28 ^{##}
G-II-Diabetic control	35.230 \pm 0.52 ^{**}	21.230 \pm 0.32 ^{**}	5.47 \pm 0.84 ^{**}
G-III Standard Drug	28.283 \pm 0.56 ^{** ##}	35.147 \pm 0.26 ^{** ##}	8.50 \pm 0.53 ^{**##}
G-IVHSE (250mg/kg)	26.422 \pm 0.73 ^{** ##}	42.244 \pm 0.75 ^{** #}	7.72 \pm 0.46 ^{** ##}
G-V HSE (500mg/kg)	22.233 \pm 0.53 ^{** ##}	45.172 \pm 0.78 ^{** ##}	8.35 \pm 0.32 ^{**##}

Values are mean \pm SEM (n=6), statistical significance, *P<0.05, **P<0.01, compared with Normal control group I; #P<0.05 ###P<0.01 compared with Diabetic control group II.

langerhans and resultant reduction of insulin release thereby inducing hyperglycemia. Insulin deficiency leads to various metabolic alterations in the animals viz-increased blood glucose, increased cholesterol, increased level of alkaline phosphate and transaminases etc. [14 -15]. The intra-peritoneal (ip) treatment with streptozotocin (dose 55mg/kg bw.) to rats significantly decreased the body weight during three weeks of treatment. The decreased in body weight in diabetic rats due to loss or degradation of structural proteins, and structural proteins are known to contribute to the body weight [16]. The administration of HSE reserved the body weight. The body weight of diabetic animals were improved significantly by the treatment of HSE (250 mg/Kg & 500 mg/Kg) may be restoration of release of protein and urinary glucose after treatment. In case of group II (diabetic control group) animals body weight and blood glucose level on the day 0 were 222.50 gm and 255.23 mg/dl that reach to 183.66 gm and 302.26 mg/dl respectively after the 21st day of treatment (Table 2 & 3).

The metformin hydrochloride was used as a standard drug. Metformin is a dimethylbiguanide oral hypoglycemic drug derived from guanidine, a hypoglycemic active compound isolated from

Galega officinalis [17]. Metformin is able to inhibit hepatic glucose production and acts as an insulin sensitizer in isolated skeletal muscle from insulin-resistant humans [18]. It was demonstrated that the enzyme AMP-activated kinase (AMPK) is activated by metformin. AMPK is a key sensor of cell energetic balance being activated by increase in the ratio AMP/ATP. There are indications that the activation of this enzyme is beneficial for the treatment and prevention of Type 2 diabetes and the metabolic syndrome, its activation leads to increased glucose uptake in rat skeletal muscle [19].

Hyperlipidemia has been reported to accompany hyperglycemia states. High levels of TC and more importantly LDL cholesterol are major coronary risk factors [20] whereas several studies showed that an increase in HDL cholesterol is associated with a decrease in coronary risk [21]. The marked increase in blood glucose and associated lipid levels that characterize the uncontrolled diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat deposits. Lowering of serum lipid concentration through drug therapy or dietary measures seems to decrease the risk of vascular diseases [22]. The effect of the administration of

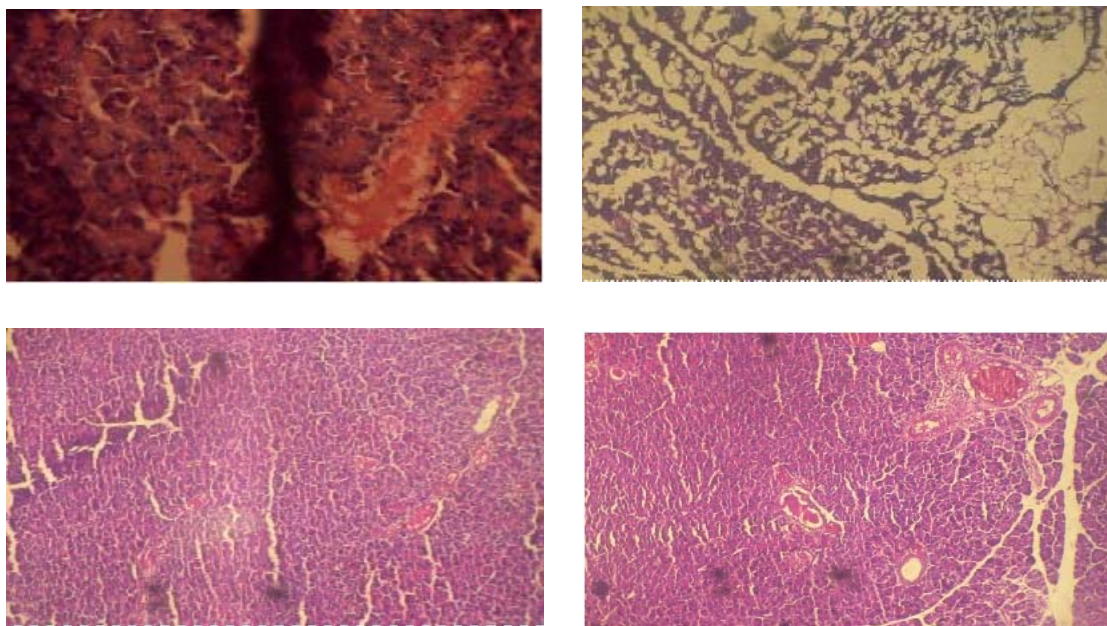


Fig. 1: Effect of HSE on rat pancreas (a. Vehicle control, b. Diabetic control, c.HSE 250 mg/kg bw. d. HSE 500 mg/kg bw.)

HSE on total lipid profile is shown in Table 4. Total cholesterol (TC) in the diabetic control group was significantly higher (230.05 mg/dl) compared to vehicle control group (162.06 mg/dl). However, TC significantly decreased to 173.32 mg/dl & 198.25 mg/dl by the treatment with the HSE 500 and 250 mg/Kg body weight respectively. Likewise, the increased TG levels were also brought down close to the normal values by administration of the HSE. The value decreased to 232.30 mg/dl & 263.45 mg/dl with the treatment of extract (500 mg/Kg & 250 mg/Kg b.w.) as compared to 302.12 mg/dl in case of diabetic control (Group II). The HDL-cholesterol was significantly decreased in the group II as compared to the healthy controls. However, treatment with the HSE caused significant improvement and increased the level to 33.12 mg/dl & 38.37 mg/dl respectively of increased dosages. Similarly, LDL values in the disease control group showed increase in 21st day of treatment. In case of HSE treated group, LDL level was significantly decreased at 102.47mg/dl and 82.23 mg/dl respectively of increased dosages as compared to group II. The standard drug treated animals (group III) also showed the significant decreased of the LDL level (85.68 mg/dl).

The increased level of lipids, TC and TG in streptozotocinized rats were observed in this experiment. Regular administration of HSE not only leads to normalization of the lipid profile of diabetic rats but also enhanced the HDL. HDL which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed 'reverse cholesterol transport', and is considered to be a cardio protective lipid [23]. A highly negative correlation between serum HDL-cholesterol level and incidences of atherosclerosis is also known to exist [24]. Thus, HSE has significant role to improving the imbalance in lipoprotein metabolism.

Table 5 showed the effect of HSE of *Tetragium angustifolia* on serum enzymes (SGOT, SGPT & ALP). It exhibits the significant reduction ($p < 0.01$) in the levels of SGOT, SGPT & ALP when compared with disease control group. Increased activity of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes, are believed responsible

for the increased gluconeogenesis and ketogenesis observed in the disease [25]. Alanine and aspartate transaminase activities are used as an indicator of hepatocyte damage [26]. Acid phosphatase activity is normally high in diseased states and is often used as a tool in clinical investigations. Data from this study show that alanine transaminase activity is elevated in diabetes, while the feeding of the HSE resulted in a significant decrease in alanine transaminase activity.

Oxidative damage has been suggested to be a contributory factor in the development and complications of diabetes. Lipid peroxidation, a type of oxidative degeneration of polyunsaturated lipids, has been implicated in a variety of pathogenic processes [27]. The elevated levels of blood glucose in diabetes produce oxygen-free radicals that cause membrane damage due to peroxidation of membrane lipids and protein glycation. The oxidative stress and resultant tissue damage are important component in the pathogenesis of diabetic complications [28]. By the oxidative stress lead to imbalance of *in-vivo* anti-oxidant system and that was evaluated by this study. The oxidative stress in the diabetic animals measured by markers since free radical measurement was difficult due to their very short half-life and their low concentrations. Therefore, indirect markers were commonly used to evaluate secondary products of lipid peroxidation such as thiobarbituric acid reactive species (TBARS) [29]. Previous studies have reported that there was an increased lipid peroxidase (LPO) in liver and kidney of diabetic rats [30]. Elevated lipid peroxidation was also studied in streptozotocinized diabetic animals. HSE and metformin HCl (Gr. IV, V & III) treated groups were significantly reduced the oxidation of lipids, the data represented on the Table 6. Glutathione (GSH) plays the important role in balance the oxidative stress. In diabetic control groups, the decreased GSH may be due to reduction in GSH synthesis or degradation of GSH by oxidation stress in diabetic animal [31]. Under *in-vivo* conditions, GSH acts as an antioxidant and its decrease was reported in diabetes mellitus [30, 32]. Here observed a significant decreased in GSH levels during diabetes. The decrease in GSH levels represents increased utilization due to oxidative stress [33]. The depletion of GSH content may also lower the glutathione S-transferase (GST) activity,

as GSH is required as a substrate for GST activity [34]. The increased GSH content in the liver and kidney of the rats treated with HSE and metformin may be one factor responsible for inhibition of LPO. Catalase (CAT) is one of the major scavenging enzymes that remove toxic free radicals *in-vivo*. Reduced activities of CAT in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of $O^{\cdot-2}$ and H_2O_2 . Catalase is haem containing ubiquities enzyme, also detoxify the H_2O_2 into water and oxygen. The level of CAT in liver and kidney was improved by treatment of HSE and standard drug. Anti-oxidants may have a role in the prevention of diabetes [32, 35]. From the above *in vivo* anti-oxidant status, it is support to anti-diabetic effect of HSE.

The presence of number of functionally active β -cells in the pancreas is an indication to improvement of diabetes (Type II). The renewal of β -cells in diabetes has been studied in several animal models. The total β -cells mass reflects the balance between the renewal and loss of these cells. The β -cells regeneration effects in STZ treated diabetic animals were reported by pancreas tonic [36], ephedrine [37], and *Gymnema sylvestre* leaf extracts [38]. In our studies, the damage of pancreas in STZ treated diabetic control rats (Fig. 1B) and regeneration of β -cells by HSE (Fig. 1C & 1D) was

observed. The histopathological studies found that HSE was non-toxic and improved for the regeneration of pancreatic cells that degraded by the effect of STZ of diabetic rats (Gr. II). Photomicrographs of pancreatic cells (Figure 1) showed normal cells with well preserved cell compactness and central vein (a). In case of group II diabetic rats, the normal lobular structure was lost. Significant fatty layer degradation has occurred. Small blackish, blood clotting spot is visible that may be sign of necrosis. (b). In Group IV (HSE 250 mg/kg), Fatty layers degradation has noticed. The layers are stretches to longitudinally long area. The duct area is prominent and ballonic present. The compactness of cells is comparatively better than diabetic control. The cell regeneration has occurred through ductus portion (c). In case of Group V (HSE 500 mg/kg), pancreatic cell compactness has managed but fatty layer fragmentation has occurred frequently. Ballonic present, scattered blood clotting has notices but cell regeneration progressively seen (d).

Thus, in conclusion the hydro-alcoholic leaf extract of *Tetragium angustifolia* (Roxb.) had significant anti-diabetic effects, improved hyperlipidemia, and other biochemical parameters. The extract had the activity to restore and regeneration of pancreatic β -cells upon treatment of diabetes (type II).

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