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EVALUATION OF ANTIHYPERGLYCEMIC AND ANTIOXIDATIVE EFFECTS OF *DILLENIA INDICA* L. USING *IN-VIVO* APPROACHES

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Keywords:

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ABSTRACT: *Dillenia indica* (DI) is a traditionally used medicinal plant which is reported to exhibit wide range of pharmacological activities. However, no vast scientific study has been conducted in this regard. The present study was designed to investigate the antihyperglycemic, antihyperlipidemic and antioxidative activities of the *Dillenia indica* fruits. Aqueous and methanolic extracts of the fruits were prepared and Phenolic and flavonoid content of the extracts were determined. Diabetic mice were prepared with alloxan (150 mg/kg) body weight (b.w.). Antihyperglycemic study was carried out with doses (150-550) mg/kg b.w. of methanolic fruit extract (MFE) in diabetic mice. Biochemical analysis of lipid profile and liver marker enzymes were carried out. Antioxidant enzymes (superoxide dismutase, catalase and glutathione reductase) activity assessments and histopathological investigations were done in liver and kidneys of mice. From the studies we found that MFE possesses higher phenolic and flavonoid content and therefore were considered for further studies. From the antihyperglycemic study it was found that 350 mg/kg b.w. dose was the most effective in reduction of blood glucose level. Significant reduction in the levels of serum lipids and liver marker enzymes and significant increase in the activities of the antioxidant enzymes were observed in the treated group. Histopathological investigation showed that detrimental effects of oxidative stress were attenuated in the treated group. It was further concluded that *Dillenia indica* fruits were found to be quite effective against hyperglycemia, hyperlipidemia and oxidative stress and therefore the fruits could be considered to be of therapeutic value in diabetes.

INTRODUCTION: Diabetes mellitus (DM), a metabolic disorder, is characterized by hyper-glycemia and insufficient secretion or action of insulin [1]. DM as considered to be the most common disorder affects almost 6% of the population in the world and the dynamics of this disease are changing rapidly in low- to middle-income countries.

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According to International Diabetes Federation's (IDF) estimates, 80% of the diabetic population across the globe will be from low and middle income countries by 2030 [2].

Mostly people with DM suffer from either type 1 diabetes (which is immune-mediated) or Type 2 DM (formerly known as non-insulin dependent DM) [3]. Type I diabetes is caused by destruction of insulin-producing beta cells in the pancreas mediated by immune system[4]. T2DM is defined by failure of the body to control blood glucose levels, which is due to impaired insulin secretion accompanied by insulin resistance [5]. DM is a chronic metabolic disease which can be clinically suspected by the onset of characteristic symptoms

such as polyuria, polydipsia, polyphagia and unsolved weight loss [6]. If not treated, being one of the greatest causes of death all over the globe results in complications associated with dysfunction, and failure of various organs[7]. Elevation of basal fat cell lipolysis during obesity is closely associated with insulin resistance [8]. Insulin resistance and disturbed lipid and glucose metabolism, results in several macro and microvascular complications [9].

It has been described that DM leads to mitochondrial electron transport chain alterations; formation of reactive oxygen species (ROS), mitochondrial energy metabolism dysfunction and ultimately oxidative stress. These are being recognized as major players in diabetes-related complications [10]. During mitochondrial oxidative metabolism, a component of utilized oxygen is reduced to water, and the remaining oxygen is transformed to oxygen free radical ($O^{\bullet}$) which is an important ROS that is converted to other species such as $ONOO^{-}$, OH and H_2O_2 [11]. ROS generated by hyperglycemia in turn cause damage to the cells in many ways which ultimately results in secondary complications in diabetes mellitus [12]. Anti-hyperglycemic drugs may have prominent detrimental effects on the body, therefore the present work has been undertaken to evaluate a particular plant used in traditional medicine.

Plants play major role in the discovery of new therapeutic agents and much attention has been received in this regard as sources of biologically active substances [13]. *Dillenia indica* L. is one such plant selected for the study. The leaf, bark, and fruit of the plant are used traditionally as medicine having good therapeutic values [14]. In the present study antihyperglycemic and antioxidative activities of the MFE of *Dillenia indica* has been ascertained in alloxan induced diabetic mice.

MATERIALS AND METHODS:

Chemicals Required: Alloxan, pyrogallol and metformin were procured from Sigma-Aldrich Co. (St. Louis, MO, USA.). Glibenclamide was purchased from Emcure Pharmaceuticals Ltd. (Pune, India). Insulin was purchased from Gland Pharma Ltd. (Hyderabad, India). SGOT, SGPT, Cholesterol, TAG kits were purchased from coral. The other chemicals used were of analytical grade procured from Merck Co. (Mumbai, India) and Sisco Research laboratory.

Plant Materials: Fruits of *Dillenia indica* were collected from Beltola area of Guwahati Assam on 15/04/2014 (Voucher No: 12056). The specimen submitted was identified by Dr. P. B. Gurung, Curator herbarium, Department of Botany, NEHU, Shillong, Meghalaya.

Experimental Animals: Healthy male Swiss albino (Balb/C strain) mice with 25 -30 gm body weight were used for the study. Mice were kept in animal house under strict laboratory conditions. All the experiments were carried out in accordance with the institutional ethics committee guidelines (Animal model) North Eastern Hill University, Shillong, India, and dated 04/12/2014. Mice were housed in polypropylene cages and maintained at a temperature of 25 ± 2 °C with a 12 h day-night cycle. They were provided a standard laboratory feed obtained from Amrut Laboratory, Pune, India, and water was fed *ad libitum*.

Preparation of Extracts: *Dillenia indica* fruits were cut into small pieces and air dried at room temperature till it dried completely and deprived of any water. The air dried fruits were powdered in a blender and extracted by continuous stirring overnight with 10 volume of methanol: aqueous solution (4:1). The mixture was filtered and the methanolic filtrate was kept in 38 degree in a rotary evaporator till it evaporates to become dry.

The dried extract was used for further experiments. For aqueous extraction the dried fruit powder was soaked in 250 ml (10 x volumes) of distilled water overnight at 25 - 30 °C with vigorous shaking in stirrer. The mixture was filtered using Whatman paper number 1.The filtrate was then lyophilized in tubes (lyophilisation) for 1-2 days [15].

Determination of Total Phenolic Content: Total phenolic content of aqueous fruit extract (AFE) and MFE of *Dillenia indica* were determined by Folin-Ciocalteu method [16].

Determination of Total Flavonoid Content: Total flavonoid content of aqueous fruit extract (AFE) and MFE of *Dillenia indica* were determined by aluminium chloride method [16].

Acute Toxicity Studies: Organisation for Economic Cooperation and Development (OECD) guidelines were followed for the toxicity studies [17]. Different concentrations of the extract were administered intraperitonially in increasing concentrations as given below:

Group A: Control mice.

Group B: 400 mg/kg b.w. dose of MFE.

Group C: 800 mg/kg b.w. dose of MFE.

Group D: 1,200 mg/kg b.w. dose of MFE.

Group E: 1,600 mg/kg b.w. dose of MFE.

Group F: 2,000 mg/kg b.w. dose of MFE.

During the study mice were fed and provided water. Animals in all groups were observed for 24 h. LD_{50} value was determined by following the arithmetic method described by Ghosh [18].

LD_{50} = Maximum dose 100% dead - product a × b / Number of animals in each group

Where a, is the dose difference and b, is the mean mortality.

Experimental Induction of Diabetes Mellitus: Overnight fasted mice (given water *ad libitum*), were administered a single high dose of alloxan (150 mg/kg) b.w. interperitonially (i.p.) prepared in citrate buffer (0.1 M, pH 4.5). After 72 hour (h) fasting blood glucose level were checked with SD check glucometer. Mice with fasting blood glucose level 200mg/dl and above were selected for further experiments.

Antihyperglycemic Study:

Long term Study: The study was performed in six different groups of overnight fasted mice consisting of six mice in each group.

Group A: Diabetic control.

Group B: Diabetic mice administered 150 mg/kg b.w. of MFE.

Group C: Diabetic mice administered 250 mg/kg b.w. of MFE.

Group D: Diabetic mice administered 350 mg/kg b.w. of MFE.

Group E: Diabetic mice administered 450 mg/kg b.w. of MFE.

Group F: Diabetic mice administered 550 mg/kg b.w. of MFE.

In long term antihyperglycemic study , MFE doses ranging from (150 - 550mg/kg) b.w. were intra-peritoneally injected to overnight fasted mice of respective groups every alternate day for a period of 21 day (d) and fasting blood glucose (FBG) level were measured on 1 d, 7 d, 14 d and 21 d respectively.

Intraperitonial Glucose Tolerance Test (IPGTT): The study was performed in five different groups of overnight fasted mice consisting of six mice in each group.

Group A: Diabetic control.

Group B: Diabetic mice administered with 350 mg/kg b.w. of the MFE.

Group C: Diabetic mice administered with metformin (200mg/kg b.w.).

Group D: Diabetic mice administered with glibenclamide (10mg/kg b.w.).

Group E: Diabetic mice administered with insulin (10U/kg b.w.).

Fasting blood glucose level was noted and the reference drugs and the extract were administered prior to glucose load (2 g/kg b.w.).The fasting blood glucose level was checked at 0.5 h, 1 h, 2 h, 4 h after the glucose load [19].

Biochemical Analysis:

Liver Marker Enzymes Determination: The study was performed in four different groups of overnight fasted mice consisting of six mice in each group

Group 1: Normal mice untreated.

Group 2: Diabetic mice untreated.

Group 3: Diabetic mice treated with 350mg/kg b.w. of MFE.

Group 4: Diabetic mice treated with 50mg/kg b.w. of vitamin C.

Blood was collected from retro-orbital sinus after 21 days of study. Serum was prepared for the test according to the protocol described by Gasting *et al.* [20] Activities of hepatic marker enzymes SGOT

and SGPT were determined by modified International Federation of Clinical Chemistry (IFCC), using Coral diagnostic kit (Coral Clinical Systems, Goa, India) and mean activities were expressed in terms of units/litre (U/L).

Lipid Profile Analysis: The study was performed in four different groups of overnight fasted mice consisting of six mice in each group.

Group 1: Normal mice untreated

Group 2: Diabetic mice untreated

Group 3: Diabetic mice treated with 350mg/kg b.w. of MFE

Group 4: Diabetic mice treated with 50mg/kg b.w. of metformin

Overnight fasted mice were anesthetized and blood was collected from retro-orbital plexus after 21 days. In lipid profile test, Total cholesterol, High density lipoprotein cholesterol (HDL-C) and triglyceride levels were estimated using CHOD/ PAP method, PEG/CHOD–PAP method, and GPO/ PAP method's kit respectively. The Low density lipoprotein cholesterol (LDL-C) and Very Low density lipoprotein cholesterol (VLDL-C) levels were calculated using the following formula.

VLDL-C = Triglycerides/5

LDL-C = Total cholesterol–(HDL-C+VLDL-C)

***In-vivo* Antioxidative Assays:** The study was performed in four different groups of overnight fasted mice consisting of six mice in each group

Group 1: Normal mice untreated.

Group 2: Diabetic mice untreated.

Group 3: Diabetic mice treated with 350mg/kg b.w. of MFE.

Group 4: Diabetic mice treated with 50mg/kg b.w. of vitamin C.

Preparation of Cytosolic and Mitochondrial Fractions of Tissues: At the end of experimental period *i.e.* 21 days, mice were sacrificed and dissected to extract kidney and liver tissues. Tissues were homogenized to make 10% (w/v) homogenates in a homogenisation medium containing 10 mM HEPES buffer, pH 7.4 containing 0.2 M mannitol, 50mM sucrose and 1 mM EDTA. Tissue homogenates were centrifuged at 1000g for 10 min at 4^{o}C and supernatant was collected and centrifuged at 7500g for10 min at 4^{o}C. The resulting supernatant from the 2nd centrifugation was collected and again centrifuged at 15000g for 10 min at 4°C.The resulting supernatant obtained was the cytosolic fraction. The resulting pellet was further washed in buffer and centrifuged at 7500g for 10 min at 4 ºC to obtain the mitochondrial fraction [21]. Total protein concentration of the cytosolic and mitochondrial fraction for the determination of the specific activities of the enzymes was determined by the Bradford method [22] using bovine serum albumin (BSA) as the standard.

Antioxidant Activity in Liver and Kidney Tissues: Activity assays of superoxide dismutase (SOD), MnSOD and CuZnSOD were performed by the method of Marklund and Marklund [23]. The change in absorbance was read at 420 nm against a blank and activity was expressed as units/mg protein.

Catalase (CAT) activity assay was performed by the method of Hugo Aebi [24]. In this assay, the rate of decomposition of hydrogen peroxide (H_2O_2) was measured at 240 nm and activity was expressed as units/min/mg protein.

Glutathione reductase was assayed according to the method of Carlberg & Mannervik [25]. The activity of the enzyme was measured at λ_{340} nm following the oxidation of NADPH. One unit of GR activity is defined as 1 µmol of NADPH oxidized per min at 37 ºC and the specific activity was expressed as units/mg protein.

Histological Investigation: After 21 d treatment, liver and kidneys were removed after sacrifice by cervical dislocation. Haematoxylin eosin (HE) staining technique was used for histological studies [26] with slight modifications. Briefly, tissues were initially fixed in formalin solution (10%) for 24 to 48 h and then fixed in 70% ethanol for future. Tissues were dehydrated in ethanol gradients (30 – 100%) and later embedded in paraffin wax blocks. The paraffin embedded tissues were trimmed into 5µm sized sections using a rotary microtome. Further, sections were fixed on glass slides and

stained with haematoxylin and eosin. Eventually, sections were mounted with DPX and observed under light microscope.

Statistical Analysis: Results are expressed as mean ± SEM for six mice in each group. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to compare differences between experimental groups using the statistical package "IBM SPSS Statistics 19.0 for Windows". Statistical significance was set at $p < 0.05$.

RESULTS: According to **Table 1**, the total phenolic content (105±.03) and flavonoid content (22 ± .001) of MFE are higher than the total phenolic (34 ± .013) and flavonoid (4 ± .001)) content of AFE in the study. Values are expressed as mean ± SEM. Gallic acid (GAE), Quercetin (QE).

TABLE 1: TOTAL PHENOLIC AND FLAVONOID CONTENT

Extract	Total phenolic content (µg Gallic acid /mg of extract)	Total flavonoid content (µg Quercetin (QE)/mg of extract)
Methanolic fruit extract(MFE)	105 ±.030	22 ±.001
Aqueous fruit extract(AFE)	34 ±.013	4 ±.001

According to **Table 2**, LD_{50}= Maximum dose (100%) death – a × *b* / n, n = number of animals in each group, a is the dose difference and b is the mean mortality; LD_{50} = 2,000 - (800/6) = 1867 mg/kg body weight. LD_{50} values of MFE are 1867 mg/kg body weight where 0% mortality was found at doses 100 mg/kg body weight and also less than 50% mortality was found in the dose of 2,000 mg/kg body weight.

TABLE 2: ACUTE TOXICITY STUDY

Groups	Doses (mg/kg)b.w.	No. of dead animals n=6	Dose difference (a)	Mean mortality (b)	Product (a*b)
1	400	0	0	0	0
2	800	0	400	0	0
3	1200	0	400	0	0
4	1600	1	400	0.5	200
5	2000	2	400	1.5	600
6				Total	800

Antihyperglycemic Studies:

Long Term Antihyperglycemic Study: The result of the long term study is depicted by **Fig. 1**. The FBG level was not reduced noticeably in group B. In group C FBG level was reduced by (32.4%) on 14d and (38.1%) on 21 d as compared to group A. Significant reduction of FBG level by (46.6%) on 14d and (63.1%) on 21 d was observed in group D. Drastic reduction of FBG level by (40%) on 7 d, (65.1%) on 14 d, (69.7%) on 21 d and (52.6%) on 7 d, (69.6%) on 14 d and (73.5%) on 21 d respectively was observed in Group E and Group F as compared to Group A in the study.

Intraperitonial Glucose Tolerance Test (IPGTT): The result of IPGTT is depicted in **Fig. 2**. Following intraperitoneal glucose load (2 g/kg b.w.), group B exhibited reduction of FBG level by (37.46%) at 1 h, (44.55%) at 2 h and (46.8%) at 4 h whereas group C exhibited significant reduction of FBG level by (47.9%) at 1 h, (58.6%) at 2h and (63%) at 4h as compared with the Group A. Noticeable reduction of FBG level was not observed in group D whereas Group E exhibited more improved glucose tolerance than other groups with significant reduction of FBG level by (60.8%) at 1 h, (83.7%) at 2 h and (87.3%) at 4 h as compared with the Group A in the study.

The result of lipid profile test is demonstrated by **Table 3**. As shown in the table, there was significant difference of total cholesterol, triglyceride, and HDL-C, LDL-C, and VLDL-C level in group 2 as compared to Group 1.

However, total cholesterol, LDL-C, VLDL-C, and triglyceride levels in Group 3 as well as in group 4 were found significantly lowered. Whereas, HDL-C level is observed to be higher than the group 2 in the study.

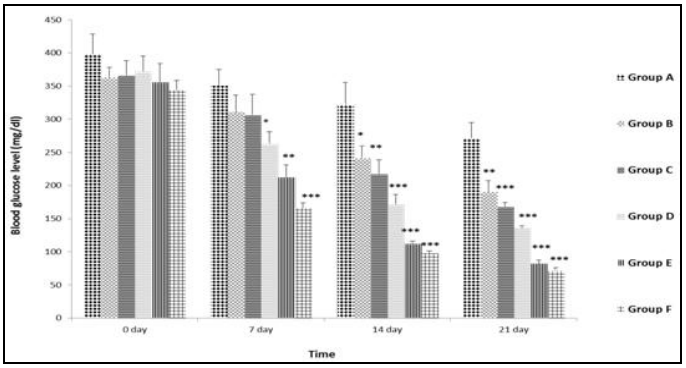

FIG 1: EFFECT OF DIFFERENT DOSES OF MFE ON FASTING BLOOD GLUCOSE LEVEL IN DIFFERENT EXPERIMENTAL GROUPS OF ALLOXAN-INDUCED DIABETIC MICE ASSAYED WEEKLY. Values are expressed as Mean ± SEM. Statistically Significant differences: * p<0.05, **p<0.01 and ***p<0.001 as compared with the control Group A

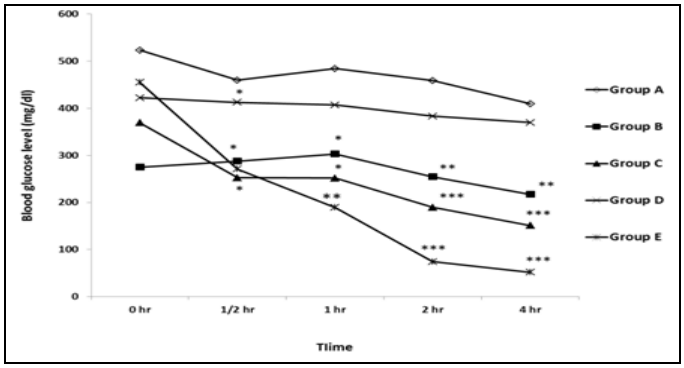

FIG. 2: RESULT OF IPGTT OF DIFFERENT EXPERIMENTAL GROUPS. The values expressed as mean±SEM; n= 6. Statistically Significant differences: * p<0.05, **p<0.01 and ***p<0.001 as compared with the Group A.

Biochemical analysis:

TABLE 3: LIPID PROFILE ANALYSIS

Groups	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Group 1	109±0.63	93.17±0.99	75.21±1.05	15.75±1.49	18.63±0.19
Group 2	166.03±1.24	136.68±0.74	49.90±0.89	88.87±1.67	27.33±0.14
Group 3	115.6±1.26***	111.12±1.42***	60.43±0.35***	33.10±1.35***	22.25±0.28***
Group 4	112±0.26***	103.80±1.27***	68.9±0.63***	23.25±0.42***	20.76±0.25**

Values are expressed as mean±SEM. Statistically Significant differences: **p<0.01 and ***p<0.001 as compared to Group 2.

Liver Marker Enzyme (SGOT) Determination: As shown in **Fig. 3**. The mean activities of SGOT were found to be significantly higher in Group 2 (107.53 ± 0.22) than in Group 1(54.49 ± 1.32), While Group 3 (73.83 ± 0.84) and Group 4 (68.16 ± 0.77) exhibited significantly lower levels of this enzyme compared to Group 2.

Liver Marker Enzyme (SGPT) Determination: As shown in **Fig. 4**, the mean activities of SGPT were found to be significantly higher in Group 2 (47.76 ± 1.69) than in Group 1 (24.19 ± 1.62), while Group 3 (31.83 ± 0.94) and Group 4 (35.31 ± 1.17) groups exhibited significantly lower levels of this enzyme as compared to Group 2.

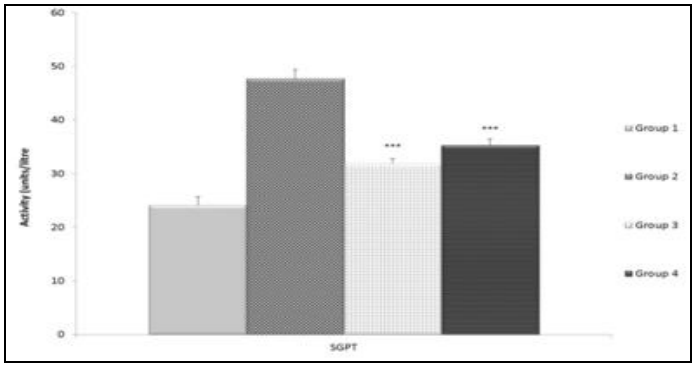

FIG. 3: MEAN ACTIVITY OF HEPATIC MARKER ENZYME SGOT IN SERUM. Values expressed as mean ± SEM, n=6. The enzyme activities are expressed as units/litre. Statistically Significant differences: ***p<0.001 as compared to Group 2.

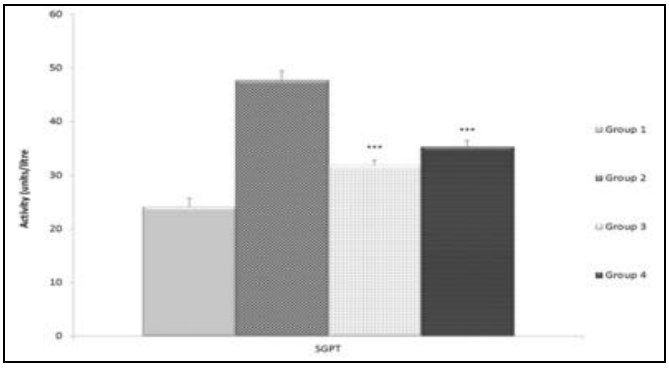

FIG. 4: MEAN ACTIVITY OF HEPATIC MARKER ENZYME SGPT IN SERUM. Values represented in mean ± SEM, n=6. The enzyme activities are expressed as units/ litre. Statistically Significant differences: ***p<0.001 as compared to Group 2 in the study.

Antioxidant Activity Determination in Liver and Kidney Tissues:

Superoxide Dismutase (SOD) Activity in Liver: As shown in **Fig. 5**, significant increase in the activities of CuZnSOD in liver was observed in Group 3 (4.33 ± 0.48) and Group 4 (4.36 ± 0.49) as compared to Group 2 (2.20 ± 0.24). Similarly significant increase in the activities of MnSOD was observed in Group 3 (11.28±0.27) and Group 4 (11.09±0.29) as compared to Group 2 (9.42±0.19) in the study.

Superoxide Dismutase (SOD) Activity in Kidney: As shown in **Fig. 6**, Increase in the

activities of CuZnSOD in kidney was observed in Group 3 (4.95 ± .20) and Group 4 (5.04 ± 0.28) as compared to Group 2 (3.07 ± 0.11). Similarly, the activity of MnSOD was found to be higher in Group 3 (10.67 ± 0.44) and Group 4 (11.41 ± 0.23) as compared to Group 2 (9.04 ± 0.25) in the study.

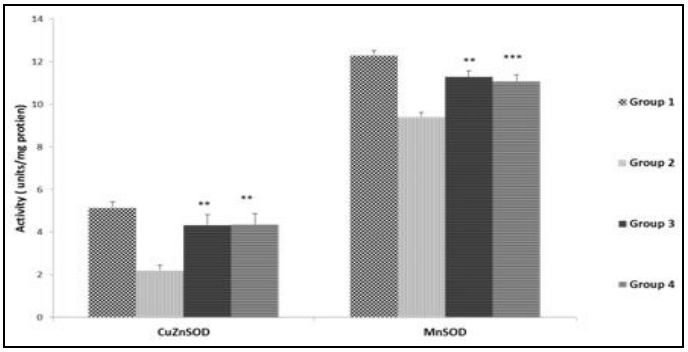

FIG. 5: MEAN ACTIVITIES OF CuZnSOD AND MnSOD IN LIVER OF MICE. Values are expressed as Mean±SEM; the enzyme activities are expressed as units/mg protein. Statistically Significant differences: **p<0.01 and ***p<0.001 as compared with Group 2

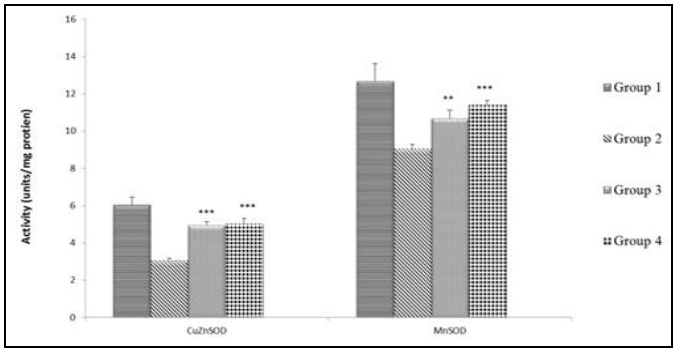

FIG. 6: MEAN ACTIVITIES OF CuZnSOD AND MnSOD IN KIDNEYS OF MICE. Values are expressed in Mean±SEM; the enzyme activities are expressed as units/mg protein. Statistically Significant differences: **p<0.01 and ***p<0.001 as compared with Group 2.

CAT Activity in Liver and Kidney: According to **Fig. 7**, the activity of catalase was observed to be higher in Group 4 (2.45 ± .14) as compared to Group 2 (1.73 ± 0.15) in liver. Whereas activity in Group 3 (3.00 ± 0.13) as compared to Group 2 and even Group 4 was observed to be significantly higher and comparable to Group 1 (3.27 ± 0.09) in the study.

In kidney, activity of catalase in Group 3 (2.29 ± 0.08) and Group 4 (2.47 ± 0.24) was observed to be significantly higher than Group 2 (1.56 ± 0.16) in the study.

Glutathione Reductase (GR) Activity in Liver and Kidney: According to **Fig. 8**, the activity of GR was observed to be higher in Group 3 (2.30 ± .55) and Group 4 (2.74 ± 0.06) as compared to Group 2 (1.73 ± 0.15) in liver. Similarly, in kidney, activity of GR in Group 3 (2.80 ± 0.20) and Group 4 (3.28 ± 0.20) was found to be significantly higher than Group 2 (1.56 ± 0.16) in the study.

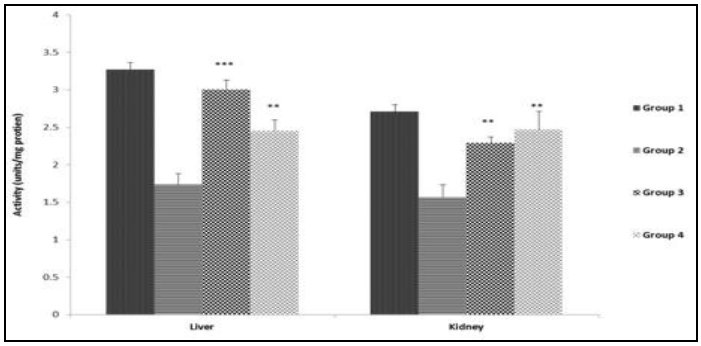

FIG. 7: MEAN ACTIVITIES OF CATALASE IN LIVER AND KIDNEY OF MICE. Values are expressed in Mean ± SEM; the enzyme activities are expressed as units/mg protein. Statistically Significant differences **p<0.01 and ***p<0.001 as compared with Group 2

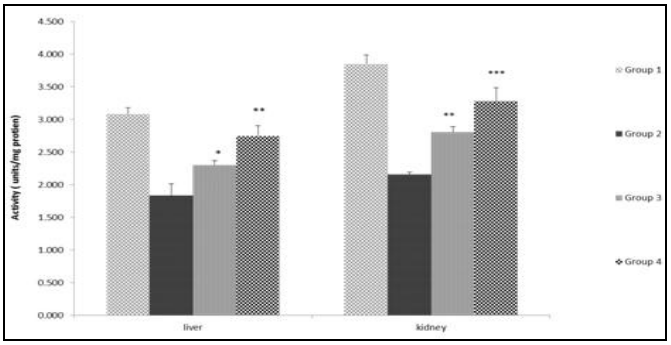

FIG. 8: MEAN ACTIVITIES OF GLUTATHIONE REDUCTASE IN LIVER AND KIDNEY OF MICE. Values are expressed in Mean±SEM; Statistically Significant differences: *p<0.05, **p<0.01 and ***p<0.001 as compared with Group 2

Histological Analysis of Liver Tissues: As shown in **Fig. 9**, normal liver tissues have normal hepatocytes with normal central vein and intact portal vein. Sinusoids were seen to have no dilation. In diabetic untreated liver tissues loss of normal architecture was observed with distended portal veins having RBC congestion and fatty vacuoles. Central veins were also observed to be distended with sinusoidal dilation and red blood cells congestion. Fewer hepatocytes were observed in diabetic untreated mice. Whereas, mild sinusoidal dilation and less distended portal and central veins were observed in the extract treated and ascorbic acid treated groups. Mild red blood cells congestion was observed.

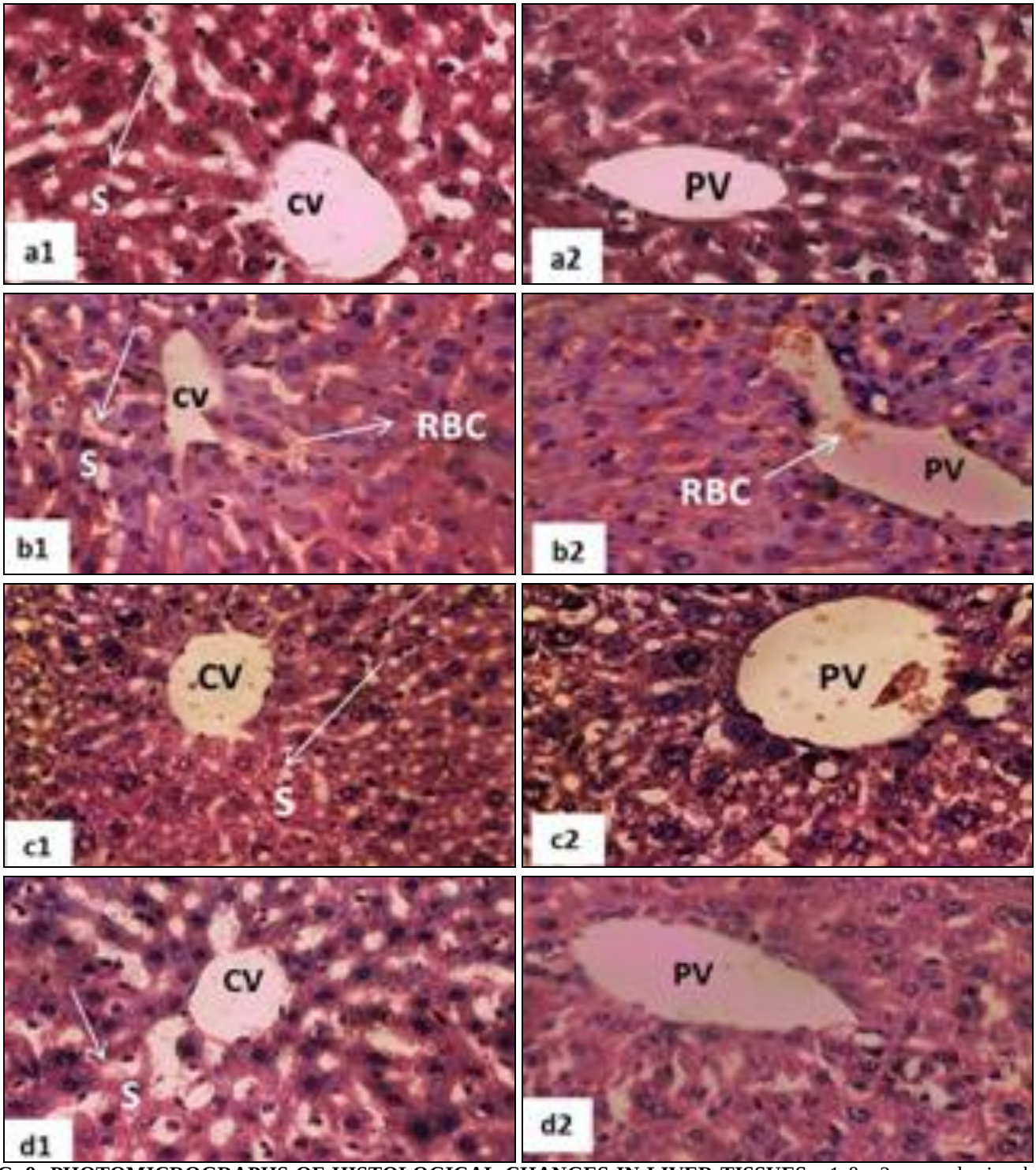

FIG. 9: PHOTOMICROGRAPHS OF HISTOLOGICAL CHANGES IN LIVER TISSUES. a1 & a2: normal mice, b1& b2: diabetic mice, c1 & c2: diabetic mice treated with MFE and d1 & d2: diabetic mice treated with the vitamin C, PV: portal veins, CV: central veins, S: sinusoids, RBC: red blood cells. All images are under 40 x magnifications.

Histological Analysis of Kidney Tissues: As shown in **Fig. 10,** normal kidney tissues shows no pathological changes in appearance showing normal architecture of glomerulus and bowman's capsular space with no inflammation in PCT and DCT. Shrunken glomerulus with widening of the bowman's capsular space and inflammation in DCT was observed in the case of diabetic kidney tissues. Less altered kidney tissues with near normal glomerular structure and without much widening of bowmen's capsular space were observed in the extract treated and ascorbic treated groups.

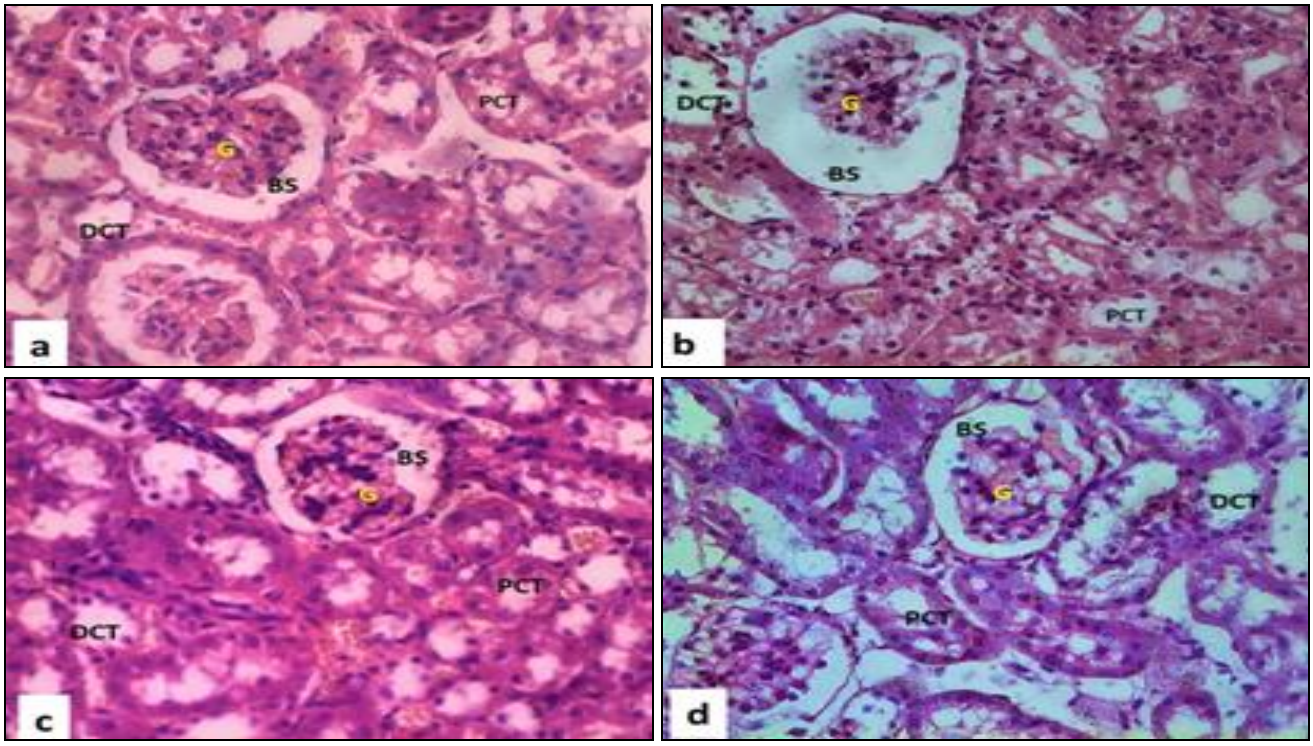

FIG. 10: PHOTOMICROGRAPHS OF HISTOLOGICAL CHANGES IN KIDNEY TISSUES. a: normal mice ,b: diabetic mice, c: diabetic mice treated with MFE and d: diabetic mice treated with the vitamin C. G: glomerulus, , BS: bowmen's capsule space, DCT: distal convoluted tubule, PCT: proximal convoluted tubule, All images are under 40x magnification.

DISCUSSION: Medicinal plants have proven their value as a source of molecules with therapeutic potential, and can represent an important pool for the identification of novel drug leads [27]. Complementary, alternative and traditional medicines are increasingly becoming popular which are commonly used to treat or prevent chronic diseases resulting in improving quality of life [28]. DI fruits were selected for the study and methanolic and aqueous extraction of the fruits was performed. Spectrophotometric phytochemical analysis conveyed that the MFE contain higher content of phenolic and flavonoids and therefore it was chosen for further studies.

Lethal dose 50 (LD_{50}) or median lethal dose which is the statistically derived single dose of a substance that produces death in 50% of a population of test animals [29]. According to the present study the acute toxicity study showed LD_{50} value of MFE to be 1867 mg/kg body weight **Table 2**. Thus, intraperitoneal administration of the extract in mice is safe and very less toxic.

The present investigation validated the anti-hyperglycemic, anti-hyperlipidemic and anti-oxidative role of DI fruits in alloxan-induced diabetic damage. It has been well documented that alloxan is most commonly used to induce diabetes in animals. Alloxan is a toxic analogue of glucose, which selectively destroys insulin-producing cells (β-cells) in the pancreas when administered to rodents [30].

In this study, significant hyperglycemia was achieved after alloxan (150 mg/kg b.w.) injection. Alloxan induced diabetic mice with more than 200 mg/dl of blood glucose level were considered to be diabetic and used for the study.

However, administration of the MFE at the dose of (150-550 mg/kg b.w.) is done to carry out long term antihyperglycemic study. In the study, FBG level was reduced in a dose dependent manner showing 350 mg/kg b.w. dose and 450 mg/kg b.w. dose of MFE to be quite effective in reduction of blood glucose level in diabetic mice, whereas, 550 mg/kg b.w. dose showed drastic reduction. Considering the entire doses 350mg/kg b.w. dose was considered as the optimum dose and used for the further study. Intraperitonial glucose tolerance study was also been carried out, where MFE and other standard drugs showed a significant effect on glucose tolerance in diabetic mice.

In extract administered group, the pattern of glucose clearance from blood was quite similar with the standard drug metformin, which acts through enhancing insulin action and absorption of glucose in peripheral tissues and inhibits gluconeogenesis. Therefore, the functional mechanism of DI in lowering blood glucose could be predicted to be similar to the mechanism of metformin's action. Lipid metabolism abnormalities observed in the context of type 2 diabetes is a major factor contributing to an increased cardiovascular risk [31]. The data shows that in diabetic condition significant elevation of plasma total cholesterol, triglycerides, LDL-C, VLDL-C, were observed. Treatment with MFE for 21 d significantly reduced total cholesterol, triglyceride, LDL-C, and VLDL-C though increase in HDL-C level can be seen indicating potent antihyperlipidemic activity.

An important contributor to postprandial glycaemic control is the net hepatic glucose uptake (NHGU) [32]. Increase in the enzymes SGOT and SGPT levels in serum with increased bilirubin level is considered to be the most relevant signal of toxicity in the liver [33]. In the present study, MFE treated mice showed lower levels of these aminotransferases compared to diabetic group **Fig. 4** and **Fig. 5**. This shows that the extract have protective roles on liver tissues against stress caused by ROS. SOD is considered a first-line of defence against reactive oxygen species. Studies have proved that with increase in blood glucose, production of ROS occurs leading to decrease of antioxidant enzymes [34]. SODs function by catalysing the reaction of disproportionation of superoxide radicals to give O_2 and H_2O_2, a reaction requiring one proton per superoxide reacted, but no external reductant [35]. Catalase and several classes of peroxidases then scavenge the H_2O_2 produced [36]. Glutathione peroxidase (Gpx), also have the similar role of detoxification of H_2O_2 to H_2O and O2 in mitochondria [37]. Subsequently, GR acts on ROS by converting GSSG to GSH and helps in maintaining a reduced intracellular environment. It was seen in the study that activity of SOD (CuZnSOD and MnSOD), CAT, and GR enzymes significantly decreased in diabetic mice as compared to normal mice. Further in the study it has been observed that MFE treated groups have shown elevated activities of antioxidant enzymes compared to diabetic control. Another important finding of the study is that DI fruits have the ability to protect and restore tissues from detrimental effect of diabetes, as evidenced by histological studies. It was found in our study that tissues under diabetic condition exhibited certain changes due to stress conditions.

The present study demonstrates that after treatment with the extract, liver and kidney tissues observed under light microscope displayed distinctly near normal architecture when compared to diabetic control **Fig. 5, 6, 7** and **8**. However, it is also clear from the study that DI fruits were able to scavenge free radicals and enhance antioxidative enzyme activities, thus allowing tissues to be protected from further impairments in diabetes which may be attributed due to the presence of phenolic and flavonoid responsible for its strong antioxidative nature.

CONCLUSION: In conclusion, the study affirms that MFE of *Dillenia indica* has more antioxidant potential with result comparable to those of the standard compounds such as vitamin C. It could also be concluded from the studies that the antioxidant activity of MFE may be due to the presence of phenolic and flavonoid content. Study confirms that the extract lowers the blood glucose level and maintains lipid profile showing its anti-hyperglycemic and anti-hyperlipidemic properties. The study also demonstrated that treatment with MFE of DI may provide beneficial effects against diabetes potentially by enhancing the levels of antioxidative enzymes and protecting the liver and kidney tissues. However, this study has not revealed the exact mechanism (s) of its action and further investigation is underway.

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