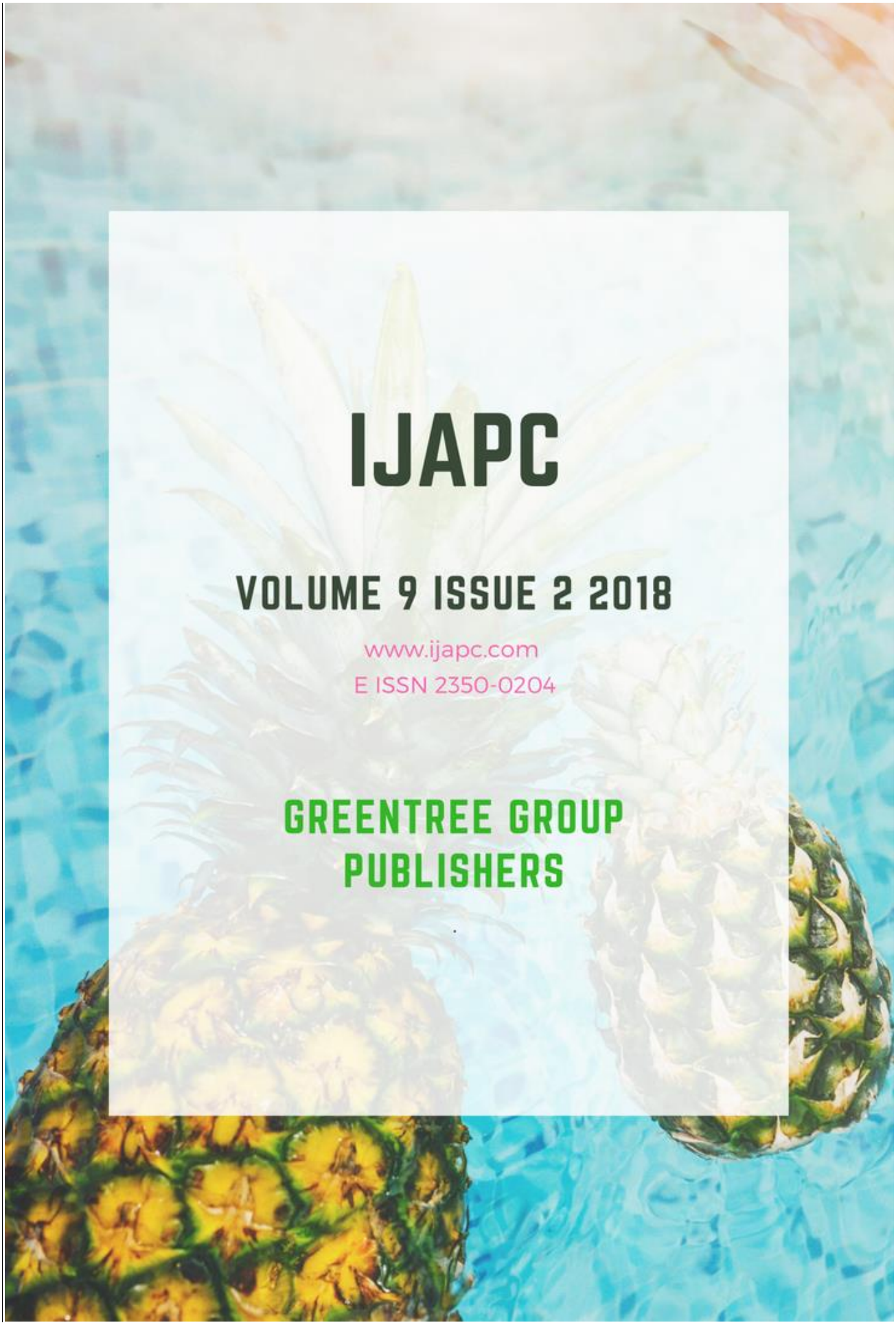

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To Study the Effect of Methanolic Extract of *Capparis decidua* (*Kair*) on Oxidative State of Liver in Diabetes Induced Wistar Albino Rats

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ABSTRACT

Type-1 diabetes is a chronic disorder characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas. The liver plays a essential role in the regulation of metabolism of carbohydrate. The core mechanism of diabetes that contributes to liver damage is the combination of increased oxidative stress and an anomalous inflammatory response. The aim of this study was to examine hepatoprotective effects of methanolic extract of *Capparis decidua* (CDMtE) against oxidative stress in streptozotocin (Stz) induced diabetic albino rats. A dose dependent effect was evaluated to elucidate relative responses of reactive oxygen species. Animals were divided into five groups i.e. Group I: Control; Group II: Diabetic control; Group III: Stz + 100 mg/kg CDMtE; Group IV: Stz + 200 mg/kg CDMtE; Group V: Negative Control treated with glibenclamide. Two different doses of *Capparis decidua* methanolic extract (i.e. 100 and 200 mg/kg body weight) were administered orally for 3 weeks. Following euthinization glycogen, glutathione, LPO, SOD and catalase were measured in liver. In this study no significant variation was observed in level of antioxidants for Group IV and Group V ($P>0.05$) when it was compared to Group I. Dose containing 200 mg/kg body weight of CDMtE showed greater ability to elevate level of antioxidants in liver. Our study showed that administration of 200 mg/kg body weight of CDMtE completely resumes level of antioxidants in liver of diabetic rats.

KEYWORDS

Medicinal plant, Type-1 diabetes, Hepatoprotective, Oxidative stress

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INTRODUCTION

Oxidation is the key process for production of energy in an organism for its utilization biological processes[1]. Over production of oxygen free radicals during oxidation amplify the oxidative stress in organisms by causing harmful effects to cell structures. Many diseased conditions like cancer, diabetes, atherosclerosis, hypertension, neurodegenerative diseases, aging, inflammation, acute and chronic liver diseases participate directly or indirectly through reactive oxygen species (ROS)[2-3].

Emergence of hyperglycemia is a common result of type-1 and type-2 diabetes, which is typically associated with excessive ROS generation and reactive carbonyl species (RCS), and/or defective antioxidant defense mechanisms. This indicates a key role of free radicals in the development and amelioration of diabetes and its pathological consequences. Overproduction of ROS and other free radicals accompanied with depletion of antioxidants defense systems lead to oxidative stress, which heighten diabetes-related complications [4].

Liver is the second largest organ in the body which is involved in vital functions like cleansing blood, vitamin synthesis, regulation of supply of body fuel, cholesterol metabolism, hormone regulation, and drug metabolism. Accumulation of liver fat in patients with type-1 diabetes has long been reported[5]. The aetiology, prevalence, and consequences of hepatic steatosis in type-1 diabetes although are not clearly understood but explains its direct linkage with oxidative stress[6].

Herbal medicines are popularized worldwide due to its ability to cure the diseases with lesser or no side effects. Ayurvedic medicine is so popular worldwide because of its effectiveness and lesser toxicity. Medicinal plants are the exceptional source of antioxidants and phytochemicals which have the ability to treat liver disorders and inflammation[7-8].

Dietary plants and herbal preparations have become increasingly attractive alternatives in the prevention and treatment of this progressive disease[9]. *Capparis decidua* (Forssk.) Edgew, commonly called Karira or Khair is one such plant that is a member of the Capparaceae family of plants and widely cultivated around South-Eastern world. The fruit of *Capparis decidua* is source of many phytoconstituents namely alkaloids, terpenoids, glycosides, phenols,flavanoids and some fatty acids. The plant has significant hypercholesterolemic, anti-inflammatory, analgesic, antidiabetic, antimicrobial,

antiplaque, antihypertensive, anthelmintic, and purgative potential [10-14].

In the present study induced diabetic Wistar albino rats were examined for hepatoprotective effects of *Capparis decidua* methanolic extract (CDMtE) against oxidative stress. A dose dependent effect was also evaluated to elucidate relative responses of reactive oxygen species.

MATERIALS AND METHODS

Tests materials:

Fresh fruits of *Capparis decidua* were procured commercially authenticated in the Department of Botany, University of Rajasthan, Jaipur, India. The fruits were shaded, dried and powdered. The powdered seeds (500 g) was extracted with 100% methanol for 72 hrs by soxhlet extraction. The extract was filtered and the solvent was separated by distillation under reduced pressure where a semi-solid dark gray crude extract was obtained (yield, 22.78%).

Animals:

Adult Wistar albino rats, approximately weighing 180–200 gm, were used in these experiments. The animals were maintained in individual polypropylene cages. The temperature in the animal house during the study period was maintained at 23 ± 2ºC. The feeding schedule comprised of two rat pellet meals a day, and water was provided *ad libitum*.

Induction of type-1 diabetes:

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared solution of 0.2 mL solution of streptozotocin (STZ) Himedia Laboratory Limited, Mumbai, India (50 mg/kg b. w. dissolved in 0.1 mM sodium citrate buffer) and pH was adjusted to 4.5 in overnight fasted rats. Control rats were introduced 0.1 mM sodium citrate buffer alone. Diabetic animals were allowed to drink 2% glucose solution overnight to overcome the drug-induced hypoglycemic shock. Animals were considered diabetic when the fasting glucose levels surpassed 250 mg/dL.

Experimental design:

Experiments were carried out three weeks (21 days) after type I diabetes had been induced, The animals were divided into five groups, five animals in each, Group I, served as control, orally received 0.5 mL distilled water each day for 21 days, Group II, served as diabetic control, Group III, Group IV were treated orally with the *Capparis decidua* extract, at a standardized dose of 100 and 200 mg/kg body weight, respectively, each day for 21 days. Group V was treated with glibenclamide (0.3 mg/kg b.w./day) dissolved in 0.5 mL distilled water and served as negative control.

Euthanization:

Animals were euthanized by cardiac puncture under ether anesthesia after the administration of last scheduled dose of extract. Liver was dissected out and preserved for biochemical tests.

Biochemical analysis:

Estimation of liver glycogen:

The phenol sulphuric acid method of Montgomery (1957)[15] was used for glycogen determinations.

Lipid peroxidation test:

Lipid peroxidation (LPO) was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in tissues by the method of Ohkawa et al (1979)[16]. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm. The values are expressed as n mol MDA/mg protein.

Estimation of glutathione:

Glutathione (GSH) was estimated according to method explained by Moron et al., (1979)[17]. Briefly, 0.2 to 1.0 ml standard Glutathione solution corresponding to 40-200µg concentration was pipetted out. The volume in all the tubes was made up to 1.0 ml with distilled water. Tissue homogenate 0.5 ml was pipetted out and precipitated with 2.0 ml of 5% TCA. 1.0 ml of supernatant was taken after centrifugation. To all these tubes 0.5 ml of Ellman's reagent was added and 3.0 ml of phosphate buffer were added. The absorbance was read at 412 nm within 2 min against the reagent blank. The amount of glutathione was expressed as n mol/g tissue.

Catalase test:

Catalase (CAT) activity was assayed according to Aebi (1984)[18]. The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of H_2O_2 consumption[19].

Superoxide dismutase estimation:

Superoxide dismutase (SOD) is estimated by the method of Marklund and Marklund (1974)[20]. The degree of inhibition of autoxidation of pyrogallol at an alkaline pH by SOD was used as a measure of the enzyme activity.

Statistical analysis:

Values are given as mean +SEM (standard error of the mean) and were compared using one way ANOVA with Tukey-Kramer multiple comparison test, to judge the difference among various groups. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Table 1 shows the concentration of LPO, GSH, glycogen. SOD and CAT in the liver of normal control and experimental groups of rats. Intraperitoneal injection of

Table1 Effect of CDMtE on oxidative stress in liver of streptozotocin induced albino rats

Sr No	Glycogen (mg/g tissue)	LPO (n mol MDA /mg protein)	GSH (n mol/g tissue)	Catalase (µ mol H_2O_2 consumed/min/mg protein)	SOD (µ mol/mg protein)
Group I	5.870±0.67	2.54±0.65	4.99±0.20	90.69±3.49	11.83±0.82
Group II	3.00±0.36***	9.69±0.96***	2.04±0.15***	40.89±3.45***	5.42±0.74***
Group III	4.93±0.97$^{\#\#ns}$	6.83±0.57$^{\#ns}$	3.97±0.06$^{\#\#ns}$	79.99±2.79$^{\#ns}$	8.63±0.82$^{\#*}$
Group IV	5.50±0.95$^{\#\#\#ns}$	3.95±0.83$^{\#\#ns}$	4.33±0.46$^{\#\#\#ns}$	80.70±3.16$^{\#\#ns}$	9.90±0.03$^{\#\#ns}$
Group V	5.71s±1.01$^{\#\#\#ns}$	2.75±0.60$^{\#\#\#ns}$	4.65±0.54$^{\#\#\#ns}$	82.87±3.88$^{\#\#\#ns}$	9.94±0.78$^{\#\#\#ns}$

ns= non-significant, *P≤0.05 compared to control, **P≤0.01compared to normal control, ***P≤0.001 compared to normal control, $^{\#}$P≤0.05 compared to diabetic control, $^{\#\#}$P≤0.01, $^{\#\#\#}$P≤0.001 compared to diabetic control

Streptozotocin to overnight fasted rats caused significant elevation in LPO level, significant reduction in levels of GSH, glycogen, SOD and CAT in diabetic control rats, as compared to normal control animal (p≤0.001) Treatment of diabetic rats with 200 mg⁄ kg CDMtE for 21 days caused a significant increase in glycogen, catalase, GSH, SOD activity, but a significant decreased in lipid peroxidation levels when compared with levels of diabetic control rats. In group III there is slight but significant changes were noted (table 1) These constituents are found to attain a near normal level in liver of oral treatment of glibenclamide (0.3 mg/kg b.w./day) in hypoglycemic rats.(group V) (figure 1).

Histology: Control rats liver section showed normal liver structure consisting of central vein and healthy hepatocytes arranged in strands(fig 2a), some of them showed double nuclei as a result of regeneration(fig.2a(i)), strands of hepatocytes separated from each other by blood sinusoids, Kuffer cells were abundant in the sinusoids (2a(i)), in diabetic rats, hepatocytes were misarranged with several areas of necrosis. Sinusoids were enlarged with the wall of veins thickened (Figure 2b). C. decidua fruit extract or glibenclamide treatments prevented these changes as evidenced by lesser signs of necrosis, lack of central hemorrhagic necrosis, mild sinusoid hyperemia, and mild connective tissue inflammation in the portal region (figure.2c-2e).

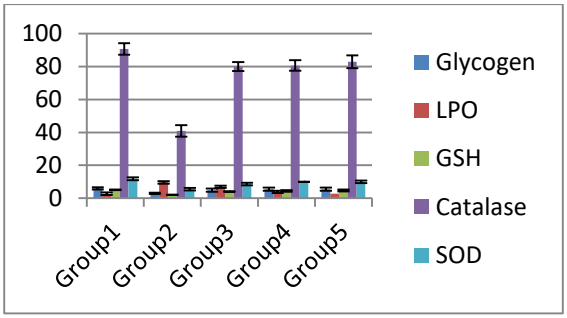

Fig 1 Group I:Control; Group II: Diabetic control; Group III: Stz + 100 mg/kg CDMte; Group IV: Stz + 200 mg/kg CDMte; Group V: Negative Control treated with glibenclamide
Values are in:
Values are in mean ± SE
Glycogen(mg/g tissue)
LPO (n mol MDA/mg protein)
GSH (n mol/g tissue)
Catalase (µ mol H_2O_2 consumed/min/mg protein)
SOD (µ mol/mg protein)

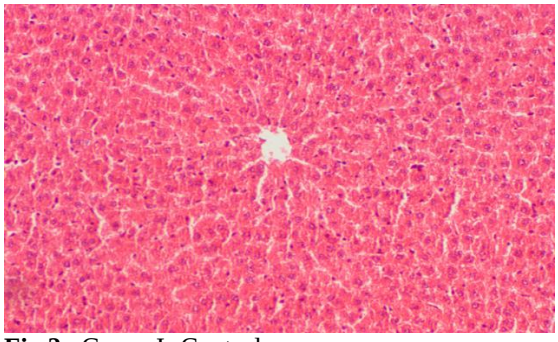

Fig 2a Group I- Control

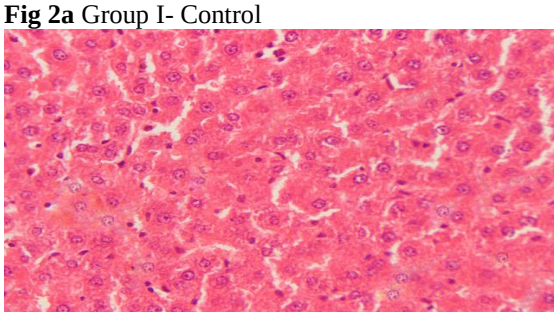

Fig 2a (i) Group I- Contro

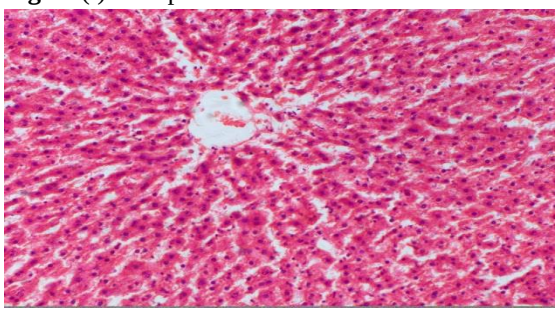

Fig 2b Diabetic control

DISCUSSION

Diabetes mellitus is characterized as a disease of carbohydrate metabolism and abnormalities of lipid and lipoprotein metabolism. Insulin deficiency stimulates lipolysis in the adipose tissue, and gives rise

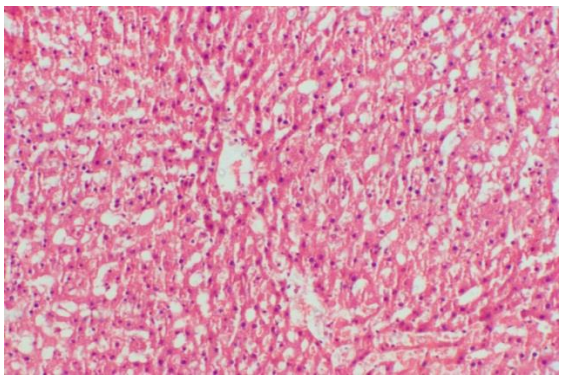

Fig 2c Group III- Stz + 100 mg/kg CDMtE

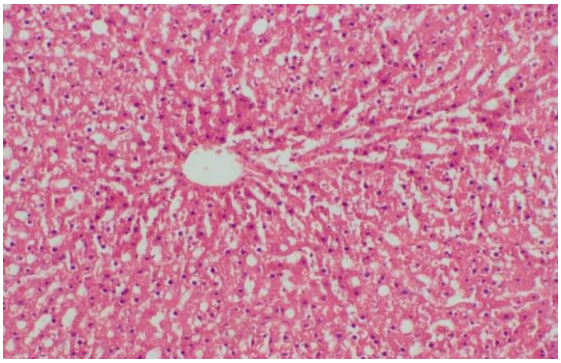

Fig 2d Group IV: Stz + 200 mg/kg CDMtE

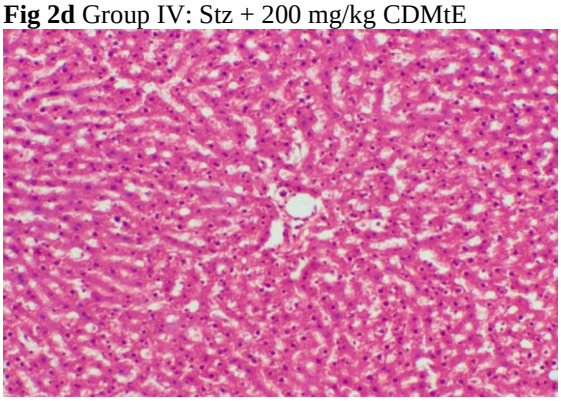

Fig 2e Group V: Negative Control treated with Glibenclamide

Figure 2: Effect of the fruits methanolic extract of C. deciduas on liver histology. Representative images of the liver in (2a) normal ,2a(i)normal control 40X (b) STZ-induced diabetic rats, (c) diabetic rats treated with 100 mg/kg/day C.decidua fruits extract, (d) diabetic rats treated with 200 mg/kg/day C. deciduas fruits extract, and (e) diabetic rats treated with 0.3 mg/kg/day glibenclamide. Images were taken under 20X magnification and 40x magnification. In diabetic rats, Photomicrography of liver section showing totally deranged general structure, several areas of moderate to severe necrosis could be seen around the central vein, dilation of central also seen. Mild to absence of necrotic changes could be seen following treatment with 200 mg/kg/day *Capparis decidua* or glibenclamide to diabetic rats.

Figure2a-2a(i); Group I- Control; 2(b); Group II- Diabetic control; 2(c); Group III- Stz + 100 mg/kg CDMtE ;2(d)-Group IV: Stz + 200 mg/kg CDMtE; 2(e)-Group V: Negative Control treated with glibenclamide

to hyperlipidemia and fatty liver in diabetes mellitus (Revan, 1988)[21]. Biochemical analysis of liver of Wistar albino rats to observe state of oxidative stress in experimental groups showed predictable

results for control, diabetic and negative (glibenclamide treated) groups.

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production. Weak defense system of the body becomes unable to counteract the enhanced ROS generation and as a result condition of imbalance between ROS and their protection occurs which leads to domination of the condition of oxidative stress[22]. Inflammatory damage that characterizes type 1 diabetes is mediated at least in part through ROS[23]. STZ destroys pancreatic β cells, resulting in a diabetic syndrome in animals, similar to that seen in human type-1 diabetes, characterized by hyperglycemia, hypoinsulinemia, glucosuria and loss in body weight[24-25]. In this study the levels of oxidative markers such as GSH, SOD, glycogen and catalase were significantly reduced in diabetic control which greatly resumed back when treated with glibenclamide (table 1). Antioxidants can act by diverse mechanisms in the oxidative sequence. The human body complex antioxidant defense system consists of the dietary intake of antioxidants, as well as the endogenous production of antioxidative compounds, such as LPO, SOD, catalase, GSH, etc.[26] Treatment of oxidative stress by herbal medicines may have some advantages[27]; as herbal medicine are the mixtures of more therapeutic or preventive components, and so might have more activity than single products alone[28-30]. Our study reports resumption of levels of Glycogen, GSH, LPO, catalase and SOD by administration of 200 mg/kg of *Capparis decidua* methanolic extract in diabetic rats (Table 1). It is to be noted that lower doses such as 100 mg/kg of *Capparis decidua* methanolic extract were also quite effective in elevating levels of antioxidant. Diabetic animals treated with 200 mg/kg CDMtE and diabetic animals treated with glibenclamide, no significant variation was visible. Although, previous study presents the determination of proximate composition, amino acids, fatty acids, tocopherols, sterols, glucosinolate and phenolic content in extracts obtained from different aerial parts of *Capparis decidua*, however, our result for the first time reports *Capparis decidua* methanolic extract as a better alternative for allopathic medicines.

CONCLUSION

It was concluded from the study that *Capparis decidua* methanolic extract has hepatoprotective effects. It greatly resumes levels of antioxidants in liver of the diabetic rats hence, protects from oxidative damages. It was also concluded from the study that CDMtE is most likely to perform

better than glibenclamide in treating type-1 diabetes, however, more evidences are required to ascertain the statement.

Animal study

The animals were maintained under veterinary supervision in accordance with the Guidelines for Care and Use of Animals in Scientific Research (INSA, 2000)[31]. All the experimental procedures and protocols used in this study were reviewed in accordance with the guidelines of the CPCSEA. (1678/GO/a/12/CPCSEA Dated 09-01-2013).

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Conflict of interest

There is no conflict of interest

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