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PROTECTIVE ROLE OF GYMNEMIC ACID IN CURBING HIGH FAT DIET AND HIGH FRUCTOSE INDUCED PANCREATIC OXIDATIVE STRESS MEDIATED TYPE-2 DIABETES IN WISTAR RATS

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Keywords:

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ABSTRACT: High fat diet and High fructose intake is the predominant lifestyle change that increased the epidemic of type-2 diabetes. HFD+HF feeding leads to oxidative stress mediated T2D through production of free radicals and decrement of antioxidant enzymes. Our present study focussed on the curbing effect of gymnemic acid on high fat diet and high fructose feeding induced oxidative stress mediated Type 2 diabetes mellitus. A total of 30 rats, 18 diabetic and 12 normal wistar rats were used for this study. Rats were HFD+HF fed and confirmed diabetic then animals were orally treated with gymemic acid (150 mg/kg body weight) daily for 30 days. Gymnemic acid supplementation effectively lowered the blood glucose and lipid levels expect HDL in HFD and HF fed diabetic rats. Free radical scavenging ability of gymnemic acid lead to the decrement of free radicals and thereby preventing macromolecular damage. Islets possess significantly lesser antioxidant potential when compared to other tissues and easily susceptible to oxidative stress under T2D condition. GPx a key antioxidant which can quench hydrogen peroxides and lipid peroxides play a crucial role in islet redox balance. Under diabetic condition GPx levels are significantly lowered. So an agent which boosts the antioxidant milieu can counter the oxidative assault effectively under pathologic condition. Gymnemic acid, an active component from the leaves of *Gymnema sylvestre* possess such boosting capacity not only at the activity level but also at the protein level. However further experimental investigation is needed to exploit its mechanism of action in increasing GPx expression is needed to substantiate its ethnomedicinal usage.

INTRODUCTION: The burden of diabetes is increasing globally, particularly in developing countries. The causes are rapid increases in overweight, including obesity and physical inactivity.

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Diabetes is predicted to become the 7th leading cause of death in the world by the year 2030, Total deaths from diabetes are projected to rise by more than 50% in the next 10 year [1].

Type 2 diabetes which is characterized by glucotoxicity and lipotoxicity leads to increased production of reactive oxygen species (ROS) and/or impaired antioxidant defence systems [2]. Islet has the lowest intrinsic antioxidant capacity compared with other metabolic tissues. Hence disturbances in the normal redox state of cells can cause toxic effects through the production of

peroxides and free radicals that damage all components of the cell, including proteins, lipids, and deoxyribonucleic acid (DNA) [3]. Hyperglycemia is the key factor behind oxidative stress of the cell. Underlying mechanism is the proton electromechanical gradient generated from the mitochondrial electron transport chain which ensuing to increased production of superoxide. Other mechanisms include glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism and sorbitol formation [4]. Similar to the paradoxically deleterious effects of chronic hyperglycemia, fatty acids (FA) and chronic hyperlipidemia, which are essential β-cell fuels in the normal state, become toxic when chronically present in excessive levels. Prolonged exposure of pancreatic β-cells causes chronic oxidative damage[5].

ROS levels need to be finely regulated to keep good radicals from going bad and thereby avoid oxidative damage to cellular processes. Antioxidant defense system plays this pivotal role in redox balance [6]. The major antioxidant enzymes are catalase, GPx, and SODs. SOD catalyzes the reaction of superoxides with hydrogen to form hydrogen peroxide. Catalase catalyzes dismutation of hydrogen peroxide to water and molecular oxygen. GPx catalyzes the reduction of hydrogen peroxide by GS and can also reduce lipid peroxides to alcohols. . GSH is regenerated for future use from its oxidized form, GSSG, through a reaction involving GSH reductase and NaDPH [7]. Malaisse *et al.*, 1982 [8] demonstrated that β-cells in albino rats were sensitive to peroxide and that the activity of GPx was low in Beta cells. Studies showed that the levels of GPx at mRNA and protein levels were low in islets and in T2D condition the expression levels are even more decreased [9].

Studies by Koulaijian and his team [10] revealed that over-expression of GPx can lessen beta cell dysfunction. Even though numerous drugs available in the treatment of diabetes, they come with the cost of potential side effects. So there is always a search for plausible drug with lesser side effects. *Gymnema sylvestre* has been extensively reported in the treatment of diabetes [11, 12, 13] and gymnemic acid, a key active constituent [14] present in the leaves accounts to 80% of this potential. Gymnemic acid has been reported to be anti-hyperglycemic [13, 15], anti-hyperlipidemic agent [16]. This present study aims whether gymnemic acid has any potential role in boosting antioxidant enzymes with special reference to GPx, a vital enzyme in quenching lipid peroxides and hydrogen peroxides.

MATERIAL AND METHODS:

Source of Chemicals: Gymnemic acid (90% pure, standardized to 75% gymnemic acid IV) was procured from St Clare herbals, Hungary. Bovine Serum Albumin (BSA) was procured from Sigma-Aldrich, USA. Enhanced chemiluminescence (ECL) kit was purchased from Millipore, USA. All other chemicals used were of analytical grade and were obtained from Medox Biotech, India, Sisco Research Laboratories Pvt., Ltd., (SRL), Genei, Bangalore and CDH (Central Drug House Pvt., Ltd., Mumbai, India).

Animals: Male Albino rats of Wistar strain were obtained from Central Animal House facility, University of Madras, Taramani campus and experiments were conducted in accordance with guidelines approved by the institutional animal ethical committee (IAEC No: 01/20/2014). The animals were housed two per cage in large spacious cages under conditions of controlled temperature (25 ± 2 °C) with 12/12 h light/dark cycle and were given food and water *ad libitum*. The animals were maintained on a commercial rat contained 5% fat, 21% protein, 55% nitrogen free extract, 4% fibre (w/w) with adequate vitamins and minerals.

Study Design: Young adult (4 months old / weighing around 130 - 150g) rats were used throughout the study. Animals were divided into five groups with six rats of each group as follows:

Groups	Particulars
Group 1	Rats fed with normal standard rat chow and water *ad libitum* served as healthy control
Group 2	Rats fed with high fat diet and high fructose (25%) in drinking water for 90 days served as diabetic control
Group 3	Rats fed with high fat diet and high fructose (25%) in drinking water for 90 days and supplemented with gymnemic acid 150 mg/kg body weight orally for the last 30 days
Group 4	Rats fed with high fat diet and high fructose (25%) in drinking water for 90 days and supplemented with metformin 50 mg/kg body weight orally for the last 30 days
Group 5	Rats fed with normal standard rat chow and water *ad libitum* and supplemented with gymnemic acid 150 mg/kg body weight orally for 30 days

High fat diet (HFD) comprised of normal rat chow supplemented with 4% cholesterol, 1.5% cholic acid and 30% coconut oil. Gymnemic acid (150 mg/kg body weight / day) was dissolved in physiological saline (0.89%) and administered by oral gavage.

Isolation of Islets: Isolation of islets is carried out based by the method of Lacy and Kostianovsky [17].

Lipid and Lipoprotein Profile: Total cholesterol (TC), triglycerides (TG) and high density lipoproteins (HDL) in serum were analysed using commercial kits from spin react in semi-auto analyser (Rx Monza, Randox, U.K).

The values of total cholesterol, triglycerides and HDL are expressed as mg/dl.

- LDL (low density lipoprotein) and VLDL (very low density lipoprotein).
- LDL cholesterol was calculated according to the Friedwald formula:
- LDL = Total cholesterol- (VLDL+ HDL cholesterol).
- VLDL (very low density lipoprotein) = TG/5
- VLDL and LDL concentrations are expressed as mg/dl.

Oral Glucose Tolerance Test and Plasma Glucose: The experimental rats were subjected to oral glucose tolerance test two days prior to sacrifice. All animals were fasted overnight and the following morning, they were subjected to oral glucose tolerance test by giving an oral dose of glucose (1 ml/100 g body weight, 50% w/v glucose solution) after collecting blood by puncturing the orbital sinus with the help of heparinized micro-haematocrit capillary tubes for estimating fasting plasma glucose and insulin. Blood samples were collected subsequently at 60, 120, and 180 min and centrifuged for 10 min at 800 x g at 4 ºC within 30 min to prevent auto-glycolysis by leukocytes. Plasma glucose was estimated by glucose oxidase-peroxidase method (Randox Laboratories Ltd.,). Results are expressed as mg/dl.

HbA1c: Glycosylated haemoglobin (HbA1c) levels were analyzed by HPLC and ion exchange method. From the blood samples collected glycosylated haemoglobin was separated from total haemoglobin. HbA1c content was calculated based on the ratio of HbA1c peak area to the total haemoglobin peak areas. Results are expressed in % glycation.

Estimation of Reactive Oxygen Species (ROS): Hydrogen peroxide, superoxide and hydroxyl radicals were estimated by the method of Jiang *et al.*, [18] Nishikimi *et al.*, [19] and Puntarulo and Cederbaum [20] respectively.

Assessment of Antioxidant Defense System: The enzyme was assayed according to the method of Marklund and Marklund [21]. The activity of catalase was assayed by the method of Sinha [22]. The activity of GPx was determined by the modified method of Rotruck *et al*., [23]. Reduced glutathione (GSH) was determined by the method of Moron *et al*., [24]

Immunoblotting: Estimation of Protein was carried out by the method of Lowry *et al.*, [25] Immunoblotting Samples containing 50 - 100 µg of proteins were separated by SDS-electrophoresis on 10%- 12% polyacrylamide gels and transferred to polyvinylidenedifluoride membranes. The membranes were incubated with specific primary antibodies and the antibodies used was GPx (CST 1:1000 dilution). To verify the uniformity of protein load and transfer efficiency across the test samples, membranes were reprobed with β-actin (CST, 1:1000 dilution). Immuno-reactive bands were developed with Immobilon Western-Chemiluminescent HRP substrate (Millipore Corporation, Billerica, USA), visualized using an enhanced chemiluminescence system (Chemi-Doc, BioRad, USA) and presented in comparison to β-actin expression

Data Analysis: The results are expressed as Mean ± Standard Error of Mean (SEM). Differences between groups were analysed by one-way analysis of variance (ANOVA) using the SPSS software package for Windows (Version: SPSS 20.0). Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD) test; significance at p value <0.05 has been given in tables and figures.

RESULTS:

Lipid Profile and FFA: Table 1 shows the impact of gymnemic acid and metformin on serum lipid profile of experimental groups. Assessment of serum lipid profile in the present study reveal that a

significant ($p < 0.05$) increase in the levels of serum total cholesterol, triglycerides, LDL, VLDL and free fatty acid (FFA) with a concomitant decrease in HDL was observed in HFD+HF alone fed group when compared to the control rats. Gymnemic acid supplementation to HFD and HF fed rats demonstrated a significant decrease ($p < 0.05$) in serum total cholesterol, triglycerides, LDL, VLDL and FFA with a concomitant increase ($p < 0.05$) in HDL, when compared to HFD+HF alone fed group, more over it was observed that gymnemic acid and metformin have almost equal beneficial effect on reducing lipid levels in the serum.

TABLE 1: LIPID PROFILE AND FFA

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
TC (mg/dl)	103.4±3.0	170.8±2.5[a]	115.5±3.0[b]	100.62±1.4[b]	104.5±2.1
TGL (mg/dl)	65.75±3.5	113.95±2.6[a]	78.03±2.2[b]	80.76±3.4[b]	68.98±1.7
LDL (mg/dl)	62.8±2.7	135.49±4.0[a]	80.2±4.0[b]	64.9±2.7[b]	64.82±1.7
VLDL (mg/dl)	13.1±0.7	22.7±0.5[a]	15.6±0.4[b]	16.1±0.6[b]	13.7±0.3
FFA (mg/dl)	231.2±4.8	343.1±12.2[a]	272.0±9.6[b]	258.3±18.9[b]	236.0±5.4
HDL (mg/dl)	27.4±1.0	12.5±1.7[a]	19.7±1.6[b]	21.5±2.4[b]	25.8±1.3

Fasting Plasma Glucose (FBG), Oral Glucose Tolerance Test (OGTT) and Glycosylated Hemoglobin (HbA1c): Fig. 1a displays fasting plasma glucose levels estimated at various intervals during experimental period. Fasting glucose levels at the initial day of initiation were around 70 - 90 mg/dl in control and experimental animals. On HFD+HF feeding rats showed 89%, 79% and 39% increase in group 2, 4 and 5 rats when compared with control rats showing a percentile increase of 9.3% at the end of 30 day period. At the end of 60 days HFD and HF fed group 2, 3 and 5 animals showed a drastic increase in the levels of the blood glucose 225 mg, 185 mg and 192 mg/dl when compared to that of the initial day 75 mg, 83 mg and 90 mg/dl respectively.

Supplementation of gymnemic acid (group 3) and metformin (group 4) from 60th for 30 days lead to the decrement of increased blood glucose levels to 95 mg and 102 mg/dl when compared to that of the diabetic control rats 282 mg/dl (group 2). No significant changes were seen between control (group 1) and drug control rats (group 5).

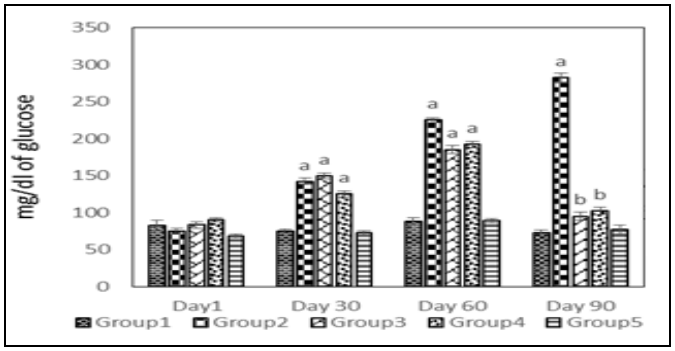

FIG 1A: FBG

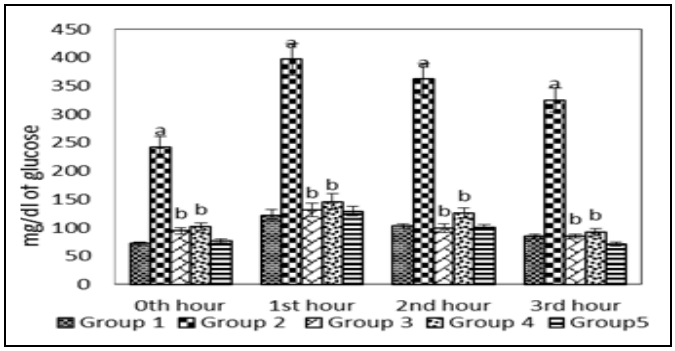

FIG 1B: OGIT

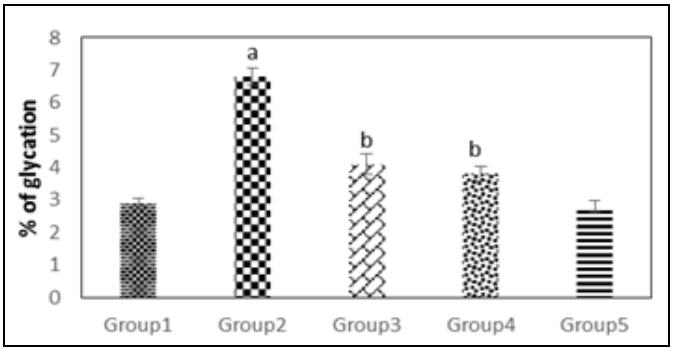

FIG 1C: HbA1c

Values are expressed as mean ± SEM for six animals in each group. 'a': control *vs* other groups, 'b': group II *vs* group III and IV, values are statistically significant at the level of $p<0.05$

Fig. 1b depicts oral glucose tolerance test (OGTT) in control and experimental animals. group 2 rats showed delayed glucose tolerance when compared to that of control animals. Group 3 and 4 rats also improved tolerance when compared to group 2 rats. Group 5 displayed a similar tolerance like group 1 control rats.

Fig. 1c shows glycosylated hemoglobin (HbA1c) levels in control and experimental animals. HbA1c levels indicated poor glycemic control in HFD and HF fed diabetic control rats (group 2). There was a 2.4 fold increase in glycation of hemoglobin in group II diabetic rats when compared to that of control rats.

Gymnemic acid and metformin supplemented groups showed 1.7 and 1.8 fold decrease in the levels of glycation when compared to diabetic control rats (group 2).

Oxidative Stress Markers:

Free Radicals: Fig. 2a, b and **c** shows the level of hydrogen peroxide, superoxide in the Islets of control and experimental rats. Group II HFD+HF fed rats showed an increase of 63% in the levels of free radicals ($p<0.05$) when compared to control animals (Group I). Administration of gymnemic acid and metformin to HFD+HF fed rats decreased the levels of free radicals generated by 32% and 30% respectively.

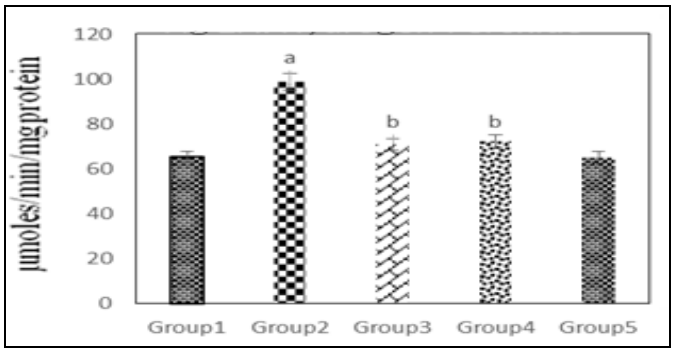

FIG. 2A: HYDROGEN PEROXIDE

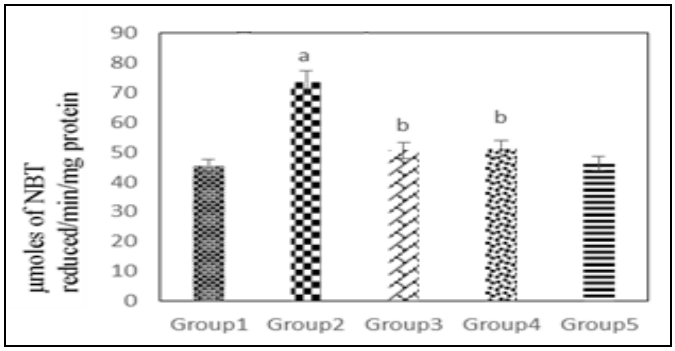

FIG. 2B: SUPEROXIDE

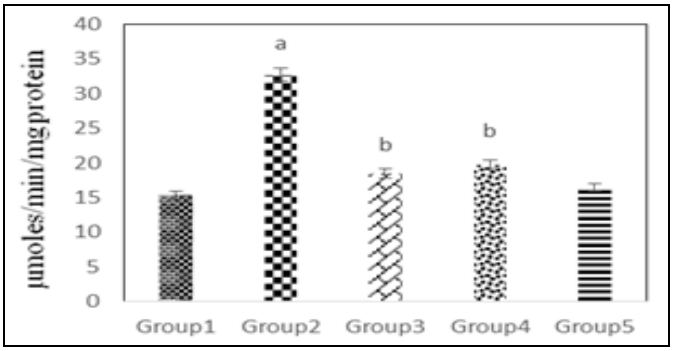

FIG. 2C: HYDROXYL

Values are expressed as mean ± SEM for six animals in each group. 'a': control *vs* other groups, 'b': group II *vs* group III and IV, values are statistically significant at the level of $p<0.05$

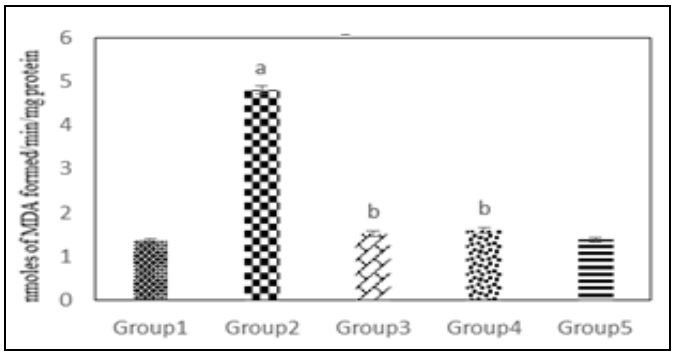

FIG. 3A: LPO

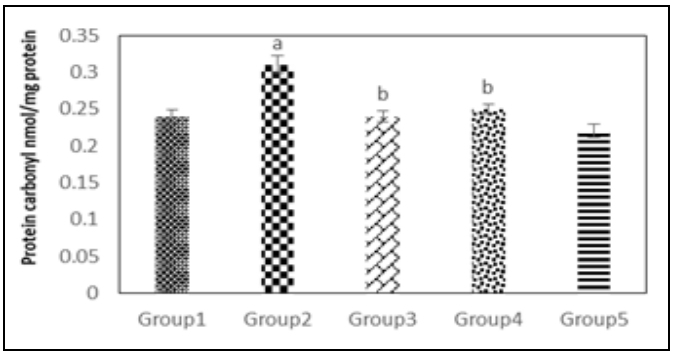

FIG. 3B: PROTEIN CARBONYL

Values are expressed as mean ± SEM for six animals in each group. 'a': control *vs* other groups, 'b': group II *vs* group III and IV, values are statistically significant at the level of $p<0.05$

Lipid Peroxide: Fig. 3a shows the effect of gymnemic acid and metformin on the levels of MDA in Islets of experimental rats. Assessment of MDA levels in experimental groups edict that MDA levels were elevated in HFD+HF fed rats due to free radical induced oxidative stress. There was a 3.5 fold increase in the MDA levels in HFD+HF fed experimental group, when compared with that of control rats. On gymnemic acid and metformin supplementation, the MDA levels were evidently brought down in HFD+HF fed rats by 3.2 and 3.0 folds respectively, when compared group 2 rats.

Protein Carbonyls: Fig. 3b shows the levels of protein carbonyls in the islets of experimental rats. Group II rats shows a significant increase (29%) in the levels of protein carbonyls when compared with control rats. Treatment with gymnemic acid and metformin significantly brought down the levels of protein carbonyls in HFD+HF fed rats by 22% and 19% correspondingly.

Enzymatic and Non Enzymatic Antioxidants: Fig. 4a - 4d shows the levels of enzymatic antioxidants in the islets of HFD+HF fed rats with or without gymnemic acid supplementation. There was a marked decrease ($p<0.05$) observed in the currently assayed antioxidant enzymes namely SOD, Catalase, GPx and GSH in HFD+HF fed rats by 37%, 30%, 39%, 41% respectively, when compared to control rats. Approximately 35-60% recovery of the activities of these enzymes have been recorded in the HFD+HF fed rats when co-supplemented with gymnemic acid. In a same way, supplementation of metformin resulted in elevation of antioxidants approximately by 15-35% respectively.

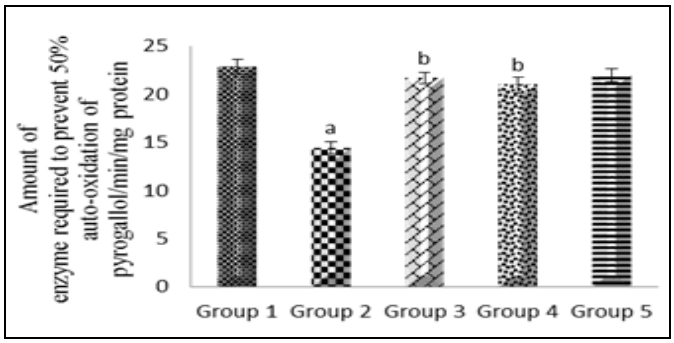

FIG. 4A: SUPEROXIDE DISMUTASE

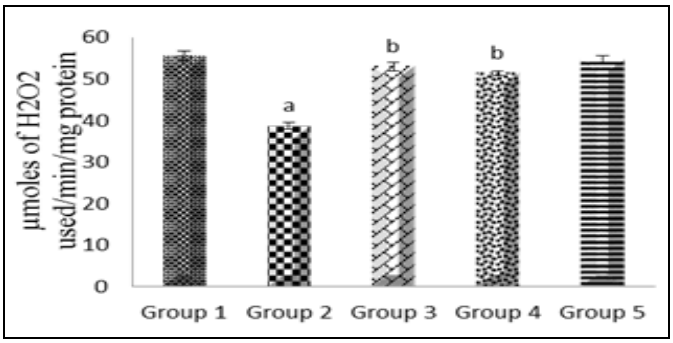

FIG. 4B: CATALASE

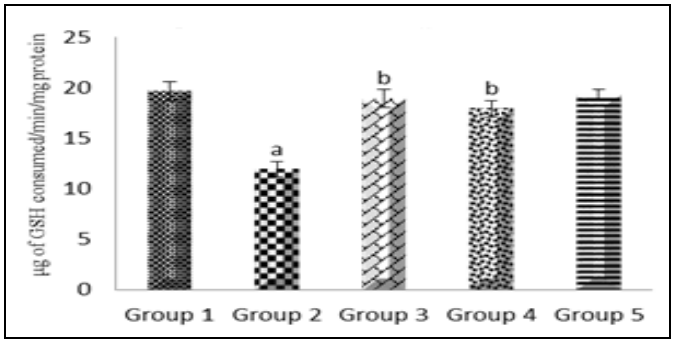

FIG. 4C: GLUTATHIONE PEROXIDASE

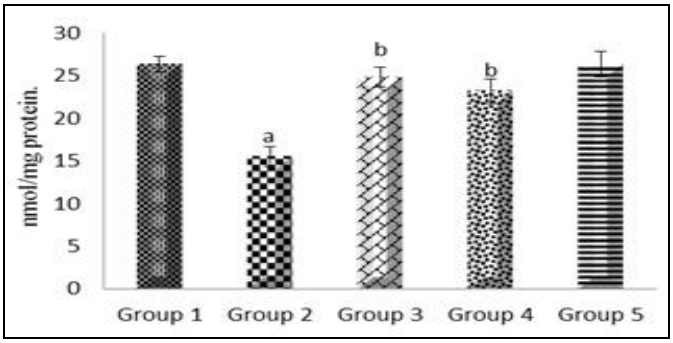

FIG. 4D: REDUCED GLUTATHIONE

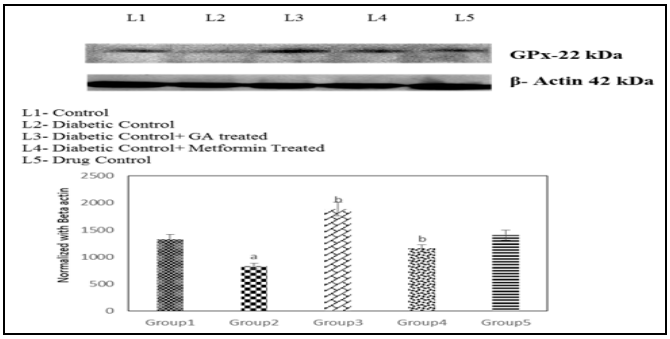

FIG. 5: GPx

Values are expressed as mean ± SEM for six animals in each group. 'a': control *vs* other groups, 'b': group II *vs* group III and IV, values are statistically significant at the level of $p<0.05$

Protein Expression of GPx: Protein expression of GPx in HFD+HF diabetic rats supplemented with or without gymnemic acid / metformin are depicted in **Fig. 5**. The present study revealed that there was a significant decrease ($p<0.05$) in protein levels of GPx in HFD+HF fed group 2 diabetic rats when compared with group 1 control rats. Upon supplementation of gymnemic acid / metformin, the protein levels of GPx were significantly increased ($p<0.05$) in diabetic rats when compared to their corresponding untreated groups.

DISCUSSION: Two major causative factors of oxidative stress in islets are lipotoxicity and glucotoxicity. Our study displays increased levels of serum lipids, FFA and lipo-proteins except HDL in HFD and HF fed T2D rats (group 2) on comparison with that to control rats. Our results are in corroboration with the previously available literature [26]. Increase in the serum lipids on feeding High fat diet and High fructose has been well documented by the studies of Jia *et al.,* [27] and Coelho *et al.,* [28]. Increase in circulatory cholesterol may be due to the presence of cholic acid in the diet. Cholic acid enhances absorption of cholesterol into the blood stream [29]. Significant restoration of altered lipid profile and circulatory free fatty acids are seen after administration of gymnemic acid to type-2 diabetic rats which is congruent with the studies of Singh *et al.,* [30] which states administration of gymnemic acid to Type-2 diabetic rats lowered the circulatory cholesterol, triglycerides, LDL-c and VLDL levels in serum and also up-regulated HDL levels. This lipid lowering activity may be attributed to its G3PDH inhibiting potential [31], intestinal fatty acid inhibitory potential [32] and prevention of partial absorption of glucose [33]. Metformin which is well attributed for its lipid curbing potential showed a slightly better reduction of circulatory lipid levels when compared with gymnemic acid treated rats.

On the other hand, our findings show a highly significant increase in blood glucose levels and impaired glucose tolerance upon high fat diet and fructose feeding for 90 days in Group 2 rats. Our results are supported by the studies of Nampurath *et al.,* [34] and Woods *et al.,* [3] which demonstrate that feeding rats with high fat diet and fructose leads to increased blood glucose and impaired glucose tolerance.

Administration of gymnemic acid to diabetic rats resulted in significant ($p<0.05$) decrease of blood glucose levels and improved glucose tolerance. Our results are coherent with the work of Sugihara *et al.,* [13] who have established the anti-diabetic activity of gymnemic acid IV in STZ induced diabetic rats. Possible mechanism of action of gymnemic acid might be mediated by its ability to inhibit the enzymes involved in the digestion of carbohydrates [36], block the absorption of glucose in the intestine [37], modulate glycolysis [31] and exert incretin mimetic activity to increase insulin secretion [38].

Glycosylated haemoglobin (HbA1c), a key bio-marker for diabetes, shows glycemic control over the past three month period. Our result shows a marked increase in the levels of glycosylated haemoglobin in group 2 rats. Increased glucotoxicity induced oxidative stress is the prime reason for glycosylation [39]. Metformin by increasing insulin sensitivity [40] reduces blood glucose levels, which in turn might have been responsible for the observed reduction in the levels of glycosylation in group 4 rats. Oral supplementation of gymnemic acid effectively lowered the levels of glycosylation in group 3 rats when compared to group 2 rats. Gymnemic acid prevents the formation of advanced glycation end products (AGEs).

This might be due to its antioxidant potential as have demonstrated its potential to scavenge free radicals in an *in vitro* study by Niramala and her co-workers[36] and Pathan *et al.,* [41]. have observed marked increase in the antioxidant status in the hearts of the cardiomyopathy induced experimental rats on supplementation with gymnemic acid-phospholipid complexes. Glucolipotoxicity leads to generation of ROS and thereby leading oxidative stress. So we sought to analyse the oxidative radicals and stress markers.

High fat diet and high fructose feeding leads to the development of type 2 diabetes and thereby to oxidative insults [42, 43]. The term "oxidative stress" is frequently used to describe the imbalances in redox status of cell, such metabolic disturbances involves the overproduction of reactive free radicals [44] or decreased levels of antioxidant enzymes [45].

Hence we measured free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals. In our study, all the free radicals demonstrated an increase in islets of the HFD+HF fed rats. Our results were in corroboration with findings of Panchal *et al.,* [46]. The products of the action of these highly reactive free radicals are molecules that are enriched in one or more oxygen atoms. These are generally considered to be markers of oxidative stress namely lipid peroxide and protein carbonyls. Protein carbonyls are formed either by direct oxidation of amino acid side chain or through conjugation by advanced lipid end products. As expected, along with increased free radicals, the lipid peroxides and protein carbonyls are also higher in HFD+HF fed rats.

Gymnemic acid has been reported to have free radical scavenging activity in an *in-vitro* study [36]. Gymnemic acid effectively scavenged the free radicals and reduced the levels of lipid peroxidation and protein carbonylation in HFD+HF fed rats. The efficiency was more or less similar to metformin. The extent of cellular damage caused by lipid peroxidation depends on the protective efficacy afforded by its innate antioxidant system [45]. Cells have a variety of enzymic and low molecular weight antioxidants as a defense against the deleterious effects of ROS overproduction and oxidative damage. Elevated ROS production, in the absence of increased antioxidant defenses would exacerbate oxidative damage and oxidative stress [47]. The biological antioxidant defense system is a unified assortment of enzymes such as SOD, CAT, GR and GPx as first line of defense and antioxidant molecules such as glutathione as second line of defense, which has been developed by the cell to protect itself against the harmful molecular species [48]. Our current findings of antioxidant defense status of experimental groups indicate, Feeding HFD+HF has been reported to reduce antioxidant enzyme activity. Treating with gymnemic acid have improved the activity of antioxidant enzymes and GSH levels, similar observations have been made by Pathan and his colleagues [41].

Since we found the considerable increase in the activity levels of GPx on supplementation of gymnemic acid to HFD+HF group than metformin, we thought to analyse the protein level expression of GPx. High fat feeding and High carbohydrate has been reported to decrease antioxidant levels and their activity.

Our findings also should similar pattern, group 2 HFD+HF fed rats showed decreased expression of GPx when compared to normal control rats. Generally GPx expression at the protein level has been reported to be very low in rodent islets. Gymnemic acid supplementation increased the expression levels of GPx there by corresponding to the increased activity of the enzyme. There are no earlier reports available to support our findings. However, Robertson *et al., (*2007) [9] reported that adenoviral overexpression of GPx increased GPx activity and protected islets against these adverse effects of ribose in HIT cell line.

CONCLUSION: The current study established that gymnemic acid supplementation was resistant to diet-induced anomalies in lipid metabolism, elevated first phase of GSIS in pancreatic islets and reduced oxidative stress in high fat diet and high fructose fed rat islets by modulating the protein expressions of GPx. These findings are important and serves as a gist because pancreatic beta cells play a key role in T2DM development. Further studies are warranted to understand the complete underlying mechanism.

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CONFLICT OF INTEREST: All the authors possess no conflict of interest in publication of this research work.

REFERENCES:

1. WHO diabetes Fact sheet November 2016.
2. Fu J, Cui Q, Yang B, Hou Y, Wang H, Xu Y, Wang D, Zhang Q and Pi J: The impairment of glucose-stimulated insulin secretion in pancreatic β-cells caused by prolonged glucotoxicity and lipotoxicity is associated with elevated adaptive antioxidant response. Food and Chemical Toxicology 2017; 100: 161-7.
3. Birben E, Sahiner UM, Sackesen C, Erzurum S and Kalayci O: Oxidative stress and antioxidant defense. World Allergy Organization Journal 2012; 5(1): 9.
4. Kassab A and Piwowar A: Cell oxidant stress delivery and cell dysfunction onset in type 2 diabetes. Biochimie 2012; 94(9): 1837-48.

5. Tangvarasittichai S: Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World journal of diabetes 2015; 6(3): 456.
6. Alfadda AA and Sallam RM: Reactive oxygen species in health and disease. BioMed Research International 2012.
7. Kurutas EB: The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition Journal 2016; 15(1): 71.
8. Malaisse WJ, Malaisse-Lagae F, Sener A and Pipeleers DG: Determinants of the selective toxicity of alloxan to the pancreatic B cell. Proceedings of the National Academy of Sciences 1982; 79(3): 927-30.
9. Robertson RP and Harmon JS: Pancreatic islet β-cell and oxidative stress: The importance of glutathione peroxidase. FEBS letters 2007; 581(19): 3743-8.
10. Koulajian K, Ivovic A, Ye K, Desai T, Shah A, George Fantus I, Ran Q and Giacca A: Overexpression of glutathione peroxidase 4 prevents β-cell dysfunction induced by prolonged elevation of lipids *in vivo*. American Journal of physiology-endocrinology and metabolism 2013; 305(2): E254-62.
11. Baskaran K, Ahamath BK, Shanmugasundaram KR and Shanmugasundaram ER: Antidiabeticeffect of a leaf extract from Gymnemasylvestre in non-insulin-dependent diabetes mellitus patients. Journal of ethnopharmacology 1990; 30(3): 295-305.
12. Nahas R and Moher M: Complementary and alternative medicine for the treatment of type 2 diabetes. Canadian Family Physician 2009; 55(6): 591-6.
13. Sugihara Y, Nojima H, Matsuda H, Murakami T, Yoshikawa M and Kimura I: Antihyperglycemic effects of gymnemic acid IV, a compound derived from *Gymnema sylvestre* leaves in streptozotocin-diabetic mice. Journal of Asian natural products research 2000; 2(4): 321-7.
14. Kurihara Y: Antisweet activity of gymnemic acid A1 and its derivatives. Life sciences 1969; 8(9): 537-43.
15. Kanetkar P, Singhal R and Kamat M: Recent Advances in Indian Herbal Drug Research Guest Editor: Thomas Paul Asir Devasagayam *Gymnema sylvestre*: A Memoir. Journal of clinical biochemistry and nutrition 2007; 41(2): 77-81.
16. Daisy P, Eliza J and Farook KA: A novel dihydroxy gymnemic triacetate isolated from *Gymnema sylvestre* possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic rats. Journal of ethnopharmacology 2009; 126(2): 339-44.
17. Lacy PE and Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967; 16(1): 35-9.
18. Jiang ZY, Hunt JV and Wolff SP: Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 1992; 202(2): 384-9.
    PubMed PMID: 1519766.
19. Nishikimi M, Appaji N and Yagi K: The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 1972; 46(2): 849-54.
    PubMed PMID: 4400444.
20. Puntarulo S and Cederbaum AI: Comparison of the ability of ferric complexes to catalyze microsomal chemiluminescence, lipid peroxidation, and hydroxyl radical generation. Arch Biochem Biophys. 1988; 264(2): 482-91.
    PubMed PMID: 2840858.
21. Marklund S and Marklund G: Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47(3): 469- 74.
    PubMed PMID: 4215654.
22. Sinha AK: Colorimetric assay of catalase. Anal Biochem. 1972; 47(2): 389-94.
    PubMed PMID: 4556490.
23. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG: Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973; 179(4073): 588-90.
    PubMed PMID: 4686466.
24. Moron MS, Depierre JW and Mannervik B: Levels of glutathione, glutathione reductase and glutathione Stransferase activities in rat lung and liver. Biochim Biophys Acta. 1979; 582(1): 67-78.
    PubMed PMID: 760819.
25. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with the Folin phenol reagent. J BiolChem 1951; 193: 265-275.
    PubMed PMID: 14907713
26. Poudyal H, Panchal S and Brown L: Comparison of purple carrot juice and β-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome. Br. J. Nutr. 2010; 104: 1322-1332.
27. Jia YJ, Liu J, Guo YL, Xu RX, Sun J and Li JJ: Dyslipidemia in rat fed with high-fat diet is not associated with PCSK9-LDL-receptor pathway but ageing. J Geriatr Cardiol. 2013; 10(4): 361-8.
    doi: 10.3969/j.issn.1671-5411.2013.04.007.
28. Coelho DF, Pereira-Lancha LO, Chaves DS, Diwan D, Ferraz R, Campos-Ferraz PL, Poortmans JR and Junior LAH: Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity, and the role of exercise on these parameters. Braz J Med Biol Res. 2011; 44(10): 966-72.
29. SenthilKumaran V, Arulmathi K, Srividhya R and Kalaiselvi P: Repletion of antioxidant status by EGCG and retardation of oxidative damage induced macromolecular anomalies in aged rats. Exp. Gerontol. 2008; 43: 176-183.
30. Singh S, Bigoniya P, Shrivastava B and Sharma J: Hypoglycemic Profile of gymnemic Acid and glycyrrhizic acid on high fructose diet related obesity induced diabetes. International Journal of Medicine and Pharmaceutical Science (IJMPS) 2016; 6(3): 61-84.
31. Ishijima S, Takashima T, Ikemura T and Izutani Y: Gymnemic acid interacts with mammalian glycerol-3-phosphate dehydrogenase. Molecular and cellular biochemistry 2008; 310(1-2): 203-8.
32. Patel SS, Shah RS and Goyal RK: Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbalayurvedic formulation in streptozotocin induced diabetic rats. Indian Journal of experimental biology 2009; 47: 564-570.
33. Shimizu H, Shimomura Y, Nakanishi Y, Futawatari A, Ohtani K, Sato N and Mori M: Estrogen increases *in vivo* leptin production in rats and human subjects. Journal of Endocrinology 1997; 154(2): 285-292.
34. Nampurath GK, Mathew SP, Khanna V, Zachariah RT, Kanji S and Chamallarnudi MR: Assessment of hypolipidaemic activity of three thiazolidin-4-ones in mice given high-fat diet and fructose. Chem. Biol. Interact. 2008; 171: 363-368.
35. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P: A controlled high-fat diet induces an obese syndrome in rats. J. Nutr. 2003; 133: 1081-1087.
36. Nirmala S, Ravichandiran V and Vijayalakshmi A: *In vitro* cytotoxicity and glucose uptake activity of gymnemic acid

fraction of *Gymnema sylvestre* leaves. International journal of pharma research and health sciences 2016; 4(2): 1104-1109.

37. Nakamura Y, Tsumura Y, Tonogai Y and Shibata T: Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in *Gymnema sylvestre* leaves. J Nutr. 1999; 129(6): 1214-22.
38. Tiwari P, Mishra BN and Sangwan NS: Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. BioMed research international 2014.
39. Giaccari A, Sorice G and Muscogiuri G: Glucose toxicity: the leading actor in the pathogenesis and clinical history of type 2 diabetes–mechanisms and potentials for treatment. Nutrition, Metabolism and Cardiovascular Diseases 2009; 19(5): 365-77
40. Giannarelli R, Aragona M, Coppelli A and Del Prato S: Reducing insulin resistance with metformin: the evidence today. Diabetes Metab. 2003; 29(4 Pt 2): 6S28-35.
41. Pathan RA, Bhandari U, Javed S and Nag TC: Anti-apoptotic potential of gymnemic acid phospholipid complex pretreatment in Wistar rats with experimental cardiomyopathy. Indian J Exp Biol. 2012; 50(2): 117-27.
42. Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, Jeandidier N, Maillard E, Marchioni E, Sigrist S and Dal S: High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. Nutrition & metabolism 2016; 13(1): 15.
43. Selvi MKN, Sridhar MG, Swaminathan RP and Sripradha R: Curcumin attenuates oxidative stress and activation of redox-sensitive kinases in high fructose- and high-fat-fed male wistar rats. Sci Pharm 2014; 83(1): 159-75
44. Hybertson BM, Gao B, Bose SK and McCord JM: Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. Mol Aspects Med. 2011; 32(4-6): 234-46.
doi:10.1016/j.mam.2011.10.006. Epub 2011 Oct 15.
45. Barrera G: Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol. Epub 2012.
46. Panchal SK, Poudyal H, Iyer A, Nazer R, Alam A, Diwan V, Kauter K, Sernia C, Campbell F, Ward L, Gobe G, Fenning A and Brown L: High-carbohydrate high-fat diet–induced metabolic syndrome and cardiovascular remodeling in rats. J Cardiovasc Pharmacol 2011; 57(1): 51-64.
doi: 10.1097/FJC.0b013e3181feb90a.
47. Filomeni G, De Zio D and Cecconi F: Oxidative stress and autophagy: the clash between damage and metabolic needs. Cell Death Differ. 2015; 22(3): 377-88.
doi: 10.1038/cdd.2014.150. Epub 2014 Sep 26.
48. Gill SS and Tuteja N: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010; 48(12): 909-30.
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