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Ameliorative effect of *Withania coagulans* on dyslipidemia and oxidative stress in nicotinamide–streptozotocin induced diabetes mellitus

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ABSTRACT

Present study aims to evaluate the effect of *Withania coagulans* fruit (aqWC) on diabetic-dyslipidemia and antioxidant/oxidant status in DM. Diabetic animals were treated with aqWC at a dose of 250 mg/kg bw for 30 days. Lipid profile, MDA, GSH, SOD, FRAP, HMG CoA reductase and acetyl CoA carboxylase activities were estimated in blood and tissues. Total cholesterol, TAG and LDL were significantly elevated whereas HDL was decreased in diabetic animals ($p < 0.05$), simultaneously the lipid content and HMG CoA reductase activities were also increased, whereas acetyl CoA carboxylase activity decreased significantly in tissues of diabetic animals. MDA was increased and antioxidants such as SOD, GSH and FRAP decreased significantly in DM ($p < 0.05$). Oral administration of aqWC to diabetic animals produced significant improvement in serum lipid profile and tissue lipid content. Activity of HMG CoA reductase decreased, whereas acetyl CoA carboxylase activity increased significantly in tissues after aqWC treatment. Administration of aqWC to diabetic animals also showed significant increase in antioxidant levels i.e., GSH, SOD, FRAP and reduced level of MDA in blood and tissue homogenates as compared to diabetic controls ($p < 0.05$). These results suggest that aqWC treatment improved lipid profile and decreased oxidative stress in diabetes mellitus.

1. Introduction

Hyperglycemia and characteristic dyslipidemia of diabetes mellitus (DM) along with increased oxidative stress leading to endothelial dysfunction have been implicated as early events in the pathogenesis of atherothrombotic macrovascular disease (Nathan et al., 1997). It is increasingly recognized that insulin deficiency contributes to the characteristic dyslipidemia associated with diabetes mellitus, and is a major risk factor for macrovascular complications (Adiels et al., 2006).

Several medicinal plants such as *Momordica charantia*, *Azadirachta indica*, *Gymnema sylvestre*, *Pterocarpus marsupim*, *Coccinia indica*, *Trigonella foenum graecum*, *Allium sativum*, *Ocimum sanctum* etc. have been reported for the management of DM and its complications. Fruit of *Withania coagulans* (Family: Solanaceae) have been reported to possess a variety of biological activities (Kirtikar and Basu, 1993) and its ethanopharmacological applications are well known (Chadha, 1976). Anti-hyperglycemic effects of fruit extract of *W.coagluans* in experimental diabetes mellitus using high doses have been reported in few studies (Hemlatha et al., 2004; Hoda et al., 2010; Jaiswal et al., 2009, 2010). Hemalatha et al. (2006) have also reported the anti-hyperlipidemic effect of aqueous extract of *W.coagulans* at a dose of 1000 mg/kg bw for 7 weeks in high fat diet induced hyperlipidemic rats. Whereas, Saxena (2010) showed the hypolipidemic activity of aqueous extract of fruit of *W.coagualns* at a dose of 1000 mg/kg bw for 28 days in streptozotocin induced diabetes. Thus, in all these reports, the doses of *W.coagulans* used were very high i.e., 750–1000 mg/kg bw/day which roughly comes to about 200 mg/rat/day and 10,000 mg/human being/day, which is higher than physiological and nutritional ranges. In our previous study, we have reported the antidiabetic/antihyperglycemic effect of aqueous extract of *W.coagulans* with low doses i.e., 250 mg/kg bw in nicotinamide–streptozotocin induced DM (Shukla et al., 2012). Hence, there is need to elucidate the beneficial effect of low doses of aqWC on dyslipidemia and modulation of various oxidant/antioxidant parameters in nicoinamide–streptozotocin induced model of DM.

2. Material and methods

2.1. Preparation of Aqueous Extract of Fruit of W.coagulans

Fruits of *W.coagulans* were purchased from the local market of Delhi and were identified and authenticated by National Institute of Science Communication and Information Resources, Pusa, New Delhi (Voucher Number, NISCAIR/RHMD/Con-

Abbreviations: ACC, acetyl CoA carboxylase; aqWC, aqueous extract of *Withania coagulans*; bw, body weight; DM, diabetes mellitus; FPG, fasting plasma glucose; FRAP, ferric reducing ability of plasma; g, gram; HMGCR, HMG CoA reductase; LDL-C, low density lipoprotein; MDA, malondialdehyde; mg, milligram; NAD, nicotinamide; GSH, reduced glutathione; SD, standard deviation; SOD, superoxide dismutase; TC, total cholesterol; TAG, triacylglycerol.

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sult/-2008–09/979/10). The fruits after removal of calyx and pedicle were soaked in distilled water and kept overnight at 4 °C. Next day, the extract was filtered through a filter paper/sterile muslin cloth to get water extract of fruit of *W.coagulans* (aqWC). Freshly prepared extract was lyophilized to get dry powder (yield 16% w/w) which was stored at 4 °C. This sticky powder was dissolved in water (1.0 ml) and fed orally to animals by intragastric tube at different doses.

2.2. Induction of diabetes in rats

Male wistar rats weighing 150 ± 10 g were housed in an air-conditioned room at temperature of 22 ± 2 °C with relative humidity 55 ± 5 units. Standard light (12 h) and dark (12 h) cycles were maintained throughout experimental period. Animals were fed with a standard laboratory diet and water *ad libitum* (Hundastan Lever Ltd., Mumbai, India). Ethical clearance was obtained from Institutional Animal Ethics Committee of Animal Research (IAEC-AR) at University College of Medical Sciences and GTB Hospital and experiments were carried out as per the guidelines of the committee.

Overnight fasted animals were made diabetic by intraperitoneal injection of nicotinamide (230 mg/kg) followed by freshly prepared streptozotocin in citrate buffer (0.1 M, pH 4.5) at a dose of 55 mg/kg bw after 15 min (Masiello et al., 1998). After 96 h of induction when blood glucose is usually stabilized, FPG was determined and rats having FPG >126 mg/dl were designated as having diabetes mellitus and were used in this experiment. No mortality has been observed in this model.

2.3. Experimental design

In our previous study, three different doses of aqueous extract of *W.coagulans* (aqWC) i.e., 125, 250 and 500 mg/kg bw were administered for 30 days which produced the significant glucose lowering effect in diabetic animals. However, 250 mg/kg bw was found to be most effective doses (MED) (Shukla et al., 2012). Therefore the present study was carried out with this dose.

The rats were divided into 4 groups ($n = 6$) as follows.

Group-I; Healthy control, Group-II; Diabetic control (Untreated), Group-III; Diabetic + aqWC (250 mg/kg bw) and Group-IV; Diabetic + Glibenclamide (0.5 mg/kg bw).

2.4. Collection of blood and tissues

Biochemical parameters in blood and tissues were determined after 30 days of aqWC treatment, whole blood (about 1.0 ml) was collected by retro-orbital venipuncture in EDTA vials. Blood was centrifuged at 1300 × g for 10 min to obtain plasma. Animal were anesthetized by single i.p. injection of pentabarbitone at a dose of 150 mg/kg bw and tissues (liver, heart and muscle) were removed, washed with cold saline and stored at −70 °C till further use for tissue constituents and enzyme assays.

2.5. Biochemical parameters

Glucose estimation was done in plasma by glucose oxidase/peroxidase method using kits (Accurex Biomedical Pvt Ltd.,). Total cholesterol (TC), triacylglycerol (TAG) and HDL-cholesterol (HDL-C) were estimated in fasting serum samples by using commercially available kits (Accurex Biomedical Pvt Ltd., Mumbai, India) and LDL-C was calculated by Friedwald's and Fredrickson's Equation. HMG-CoA reductase activity (HMGCR) was estimated by the method of Rao and Ramakrishnan (1975). HMG-CoA and mevalonate levels in liver, heart and muscle homogenates were estimated by colorimetry and the ratio of two was taken as an index of activity of enzyme, decreased ratio indicated increased activity and vice versa. Acetyl CoA carboxylase activity (ACC) in liver, heart and muscle was estimated by the methods of Numa (1960) and Nakanishi and Numa (1970). Estimation of reduced glutathione (GSH) in whole blood and tissue homogenates was carried out by the method of Beutler et al. (1963) and Ellman (1959) respectively by using dithio-nitrobenzene (DTNB). The extent of lipid peroxidation (MDA) in serum and tissue homogenates was estimated by measuring the thiobarbituric acid reactive substance (TBARS) as described by of Satoh (1978) and Wills (1966) respectively. Activity of superoxide dismutase (SOD) was measured in erythrocytes and tissue homogenates by the method of Marklund and Marklund (1974) as modified by Nandi and Chatterjee (1988). Total anti-oxidant capacity of plasma was determined by measuring the ability to reduce Fe^{3+}–Fe^{2+} (FRAP), which was estimated by the method of Benzie and Strain (1999). Total lipids from tissues were extracted and estimated by the method of Folch et al. (1957).

2.6. Statistical Analysis

Data were expressed as mean ± SD. Total 6 animals were included in each group and experiments were performed in duplicate. The data were analyzed by repeated analysis of ANOVA followed by Turkey's test using SPSS 17 software. The significance of results were considered at $p < 0.05$.

3. Results

3.1. Effect of aqWC on plasma glucose levels

The antidiabetic and anti-hyperglycemic effect of aqueous extract of *W.coagulans* has already been reported in our previous study. However in brief, the diabetic animals were divided and fed three different doses of aqWC i.e., 125, 250 and 500 mg/kg bw for 30 days. Significant anti-hyperglycemic effect was observed with all the three doses however, 250 mg/kg bw showed maximum effect on fasting and postprandial plasma glucose of diabetic animals ($p < 0.05$) and no extra benefit was observed with higher dose. Therefore, further experimental work was carried out with this dose (Shukla et al., 2012).

3.2. Effect of aqWC on serum lipid profile

Total cholesterol, TAG and LDL-C significantly increased in diabetic animals as compared to healthy controls. Diabetic animals-treated with aqWC showed significant decrease in TC, TAG, and LDL-C levels as compared to diabetic controls. HDL-C levels were lower in diabetic animals and treatment with aqWC increased HDL-C levels significantly. Thus, treatment with aqWC improved the lipid profile parameters of diabetic animals ($p < 0.05$). The results were compared with standard drug i.e., glibenclamide treated-diabetic animals, which also showed significant improvement in lipid profile (Table 1).

3.3. Effect of aqWC on regulatory enzymes of lipid metabolism

Activities of HMGCR and ACC were assayed in liver, heart and muscle tissue homogenates. HMGCR activity was significantly increased in diabetic animals as the ratio of HMG CoA/mevalonate decreased significantly as compared to healthy animals ($p < 0.05$). However, diabetic animals treated with aqWC for 30 days showed decrease in the activity of HMGCR as compared to diabetic animals. Acetyl CoA carboxylase activity was significantly decreased in diabetic animals as compared to healthy animals. However, diabetic animals treated with aqWC for 30 days showed significantly increased ACC activity as compared to diabetic controls ($p < 0.05$) (Table 2).

3.4. Effect of aqWC on total lipid content extracted from tissues

Total lipid contents of liver, heart and muscle tissue were significantly higher in diabetic animals as compared to healthy animals ($p < 0.05$). However, treatment of diabetic animals with aqWC showed significantly reduced lipid content in these tissues as compared to diabetic animals ($p < 0.05$). Glibenclamide treated diabetic animals also showed significant decrease in total lipid content in liver, heart and muscle tissue as compared to diabetic controls (Fig 1).

3.5. Effect of aqWC on oxidant/antioxidant levels in blood

In diabetic animals, the MDA levels in serum were significantly increased as compared to healthy controls ($p < 0.05$), however, aqWC treatment for 30 days showed significantly reduced MDA as compared to diabetic controls ($p < 0.05$). Glibenclamide treated diabetic animals also showed significant decrease in MDA levels as compared to diabetic controls. Reduced glutathione (GSH) and SOD activity in diabetic animals were significantly decreased as compared to healthy controls ($p < 0.05$). Whereas, aqWC treated and glibenclamide treated diabetic animals had significantly increased levels of GSH and SOD activity ($p < 0.05$). FRAP, a measure of total antioxidant capacity in plasma, it was significantly decreased in diabetic controls as compared to healthy controls

Table 1
Effect of aqWC treatment on lipid profile in different groups.

Group	Total cholesterol (TC) (mg/dl)	Triacylglycerol (TAG) (mg/dl)	High density lipoprotein (HDL) (mg/dl)	Low density lipoprotein (LDL) (mg/dl)
Healthy control	72.2 ± 9.8	97.4 ± 4.4	33.9 ± 2.3	18.8 ± 6.4
Diabetic control	126.4 ± 7.4[a]	153 ± 8.6[a]	23.1 ± 0.6[a]	72.0 ± 5.0[a]
Diabetic + aqWC (250 mg/kg bw)	85.4 ± 7.2[b, a]	96.6 ± 2.7[b]	30.2 ± 1.4[b]	36.0 ± 5.3[b, a]
Diabetic + Glibenclamide (0.5 mg/kg bw)	93.2 ± 6.0[b, a]	101.6 ± 5.5[b]	29.3 ± 1.3[b]	43.6 ± 3.6[a]

[a] $p < 0.05$ vs. healthy control.
[b] $p < 0.05$ vs. diabetic control, $n = 6$.

Table 2
Effect of aqWC treatment on lipid metabolizing enzymes in liver, heart and muscle tissue.

Group	HMGCoA/mavalonate ratio[c]			Acetyl CoA carboxylase (U/mg protein)		
	Liver	Heart	Muscle	Liver	Heart	Muscle
Healthy control	3.72 ± 0.4	2.47 ± 0.2	3.11 ± 0.6	0.18 ± 0.054	0.11 ± 0.002	0.13 ± 0.030
Diabetic control	2.45 ± 0.2[a]	1.08 ± 0.2[a]	1.90 ± 0.4[a]	0.07 ± 0.008[a]	0.02 ± 0.001[a]	0.06 ± 0.005[a]
Diabetic + aqWC (250 mg/kg bw)	3.12 ± 0.4[b, a]	2.30 ± 0.3[b]	2.93 ± 0.6[b]	0.15 ± 0.011[b]	0.10 ± 0.003[b]	0.09 ± 0.010[b]
Diabetic + Glibenclamide (0.5 mg/kg bw)	2.95 ± 0.5[b, a]	1.94 ± 0.2[b, a]	2.49 ± 0.4[b, a]	0.16 ± 0.072[b]	0.09 ± 0.003[b]	0.08 ± 0.010 [b, a]

[a] $p < 0.05$ vs. healthy control.
[b] $p < 0.05$ vs. diabetic control, $n = 6$.
[c] The activity of HMG CoA reductase is expressed by the ratio of HMG CoA/Mevalonate which is inversely proportional to its activity.

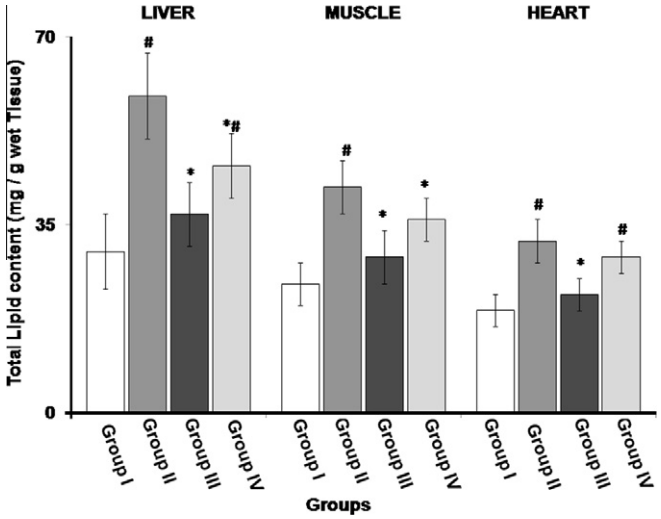

Fig. 1. Effect of aqWC treatment on total lipid content in liver, heart and muscle. Group I; healthy control, Group II; diabetic control, Group III; Diabetic + aqWC (250 mg/kg bw), Group IV; Diabetic + Glibenclamide (0.5 mg/kg bw). #$p < 0.05$ vs. Group I, *$p < 0.05$ vs. Group II, $n = 6$.

($p < 0.05$). Treatment with aqWC or glibenclamide to diabetic animals produced significant increase in FRAP ($p < 0.05$) (Table 3).

3.6. Effect of aqWC on oxidant/antioxidant levels in tissue homogenates

In diabetic animals, the GSH and SOD reduced whereas MDA increased significantly ($p < 0.05$) in liver, heart and muscle as compared to healthy animals ($p < 0.05$). However, treatment with aqWC showed significant improvement in antioxidant status as shown by increased levels of GSH and SOD activity, whereas MDA decreased in Group III ($p < 0.05$) (Fig 2).

4. Discussion

Diabetes mellitus is a metabolic disorder due to decreased insulin secretion or insulin resistance, with common feature of hyperglycemia. In the present study, diabetes was induced by single i.p. injection of nicotinamide followed by streptozotocin. Streptozotocin generates free radicals which break the DNA strands, resulting in the activation of the PARP and depletion of intracellular NAD, which appear to be common factors in β-cell death, generally leading to type I diabetes. Therefore, NAD supplementation protects against β-cells damage and helps in creating a model which is quite similar to type 2 DM (Masiello et al., 1998). In our earlier study we have reported that treatment of diabetic animals with aqWC for 30 days showed significant decrease in fasting and postprandial glucose as well as modulated the activities of enzymes of glucose homeostasis (Shukla et al., 2012). These results are comparable

Table 3
Effect of aqWC treatment on Lipid peroxidation, reduced gutathione, superoxide dismutase and ferric reducing ability of plasma.

Group	Plasma		Hemolysate	
	Lipid peroxidation (MDA) (nmol/ml)	Ferric reducing ability of plasma (FRAP) (mmol/L)	Reduced glutathione (GSH) (mg/g Hb)	Superoxide dismutase (SOD) (U/g Hb)
Healthy control	1.18 ± 0.3	2.85 ± 0.74	6.70 ± 0.8	2012 ± 88
Diabetic control	5.65 ± 0.7[a]	1.02 ± 0.25[b, a]	2.09 ± 0.5[a]	1302 ± 101[a]
Diabetic + aqWC (250 mg/kg bw)	2.72 ± 0.8[b, a]	1.96 ± 0.30[b, a]	4.08 ± 0.6[b,a]	1762 ± 73[b,a]
Diabetic + Glibenclamide (0.5 mg/kg bw)	3.09 ± 0.6[b, a]	1.74 ± 0.44[b, a]	4.14 ± 0.7[b,a]	1785 ± 106[b,a]

[a] $p < 0.05$ vs. healthy control.
[b] $p < 0.05$ vs. diabetic control, $n = 6$.

with glibenclamide treatment which also showed significant decrease in fasting and postprandial plasma glucose levels.

In Diabetes mellitus, the lipid metabolism is altered and there is increased mobilization of free fatty acids from muscle and fat deposition in tissues like liver and heart (Bloomgarden, 2003). The typical dyslipidemia of DM is characterized by increased triacylglycerol (TAG) and decreased high density lipoprotein (HDL), in addition to hypercholesterolemia which is a major risk factor for atherosclerosis and cardiovascular disease (Mooradian, 2009). Insulin affects many sites of lipid metabolism and regulates cholesterol and fatty acid biosynthesis by regulating the lipid metabolizing enzymes (Murray et al., 2006). The aqWC treated diabetic animals showed significantly decreased TC, TAG and LDL-C levels and increased HDL-C, which may help to reduce the risk of diabetes associated complications. Treatment with aqWC also showed significant decrease in lipid content in liver, heart and muscle as compared to diabetic animals.

Acetyl CoA carboxylase plays an essential role in regulating fatty acid biosynthesis, when a cell or organism has more than enough metabolic fuel to meet its energy needs, the excess is generally converted to fatty acids and stored as lipids such as TAG (Murray et al., 2006). The reaction catalyzed by acetyl-CoA carboxylase is the rate-limiting step and important site of regulation of fatty acid biosynthesis. Insulin stimulates fatty acid synthesis by activating this enzyme (Witters et al., 1988). In diabetes mellitus, the activity of ACC decreased significantly, whereas after treatment with aqWC, the activity of ACC was significantly increased in treated-diabetic animals. That effect might be due to overall improvement in glycemic control after aqWC treatment in diabetic animals.

HMGCoA reductase (HMGCR) is a polytropic, transembrane protein and an important regulatory enzyme of cholesterol biosynthesis. It stimulates the production of mevalonic acid from HMGCoA. In DM, the activity of HMGCR is increased due to high plasma glucose levels. Elevated plasma glucose and insulin levels promotes the activity of HMGCR in tissues but several studies have reported that moderate insulin deficiency or plasma glucose <360 mg/dL do not have any significant effect on the activity of HMGCR (Young et al., 1982). HMGCR activity was decreased in aqWC treated-diabetic animals which may be due to decreased availability of substrate i.e., acetyl CoA, which may be diverted towards fatty acid biosynthesis and ultimately decrease cholesterol biosynthesis.

In our previous study, we reported that aqWC has bioactive plant metabolites i.e., alkaloids, flavonoids, terpenoids, saponins, glycosides, steroidal compounds, saponins, phenols and tannins. These phytochemicals are potential antioxidants and free radical scavengers (Kusirisin et al., 2009; Georgetti et al., 2003).

Oxidative stress results from imbalance between the production of free radicals and antioxidant defense mechanisms and is an important causative factor in several chronic diseases viz diabetes and associated complications (Halliwell, 1994). Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species (Memisoğullari et al., 2003). The increased level of lipid peroxidation induces oxidative damage by increasing peroxy radicals and hydroxyl radicals. Flavonoids present in aqWC are effective in reducing lipid peroxidation and may enhance the antioxidant enzyme activity resulting the decreased level of MDA. This antioxidant potency of flavonoids could be due to arrangement of hydroxyl groups on benzene ring (Van Acker et al., 1996). Hyperglycemia can induce oxidative stress through advanced glycation end product (AGEs) formation and increased polyol and hexosamine pathway (Chakravarthy et al., 1998). AGEs produce ROS and superoxide and the subsequent increase in oxidative stress may lead to endothelial dysfunction and ultimately cardiovascular disease (CVD) through several different mechanisms. (Creager et al., 2003) Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide (H_2O_2) which is then transformed into water. Hydrogen peroxide can also give rise to hydroxyl radicals in the cells. Thus the removal of H_2O_2 is very important for antioxidant defense in cell or food systems. H_2O_2 can cross membranes and may oxidize a number of compounds. AqWC treated diabetic animals showed improved SOD activity which may be attributed to the antioxidant present in aqWC, but importantly due to decrease glucose levels (Kusirisin et al., 2009). Reduced glutathione

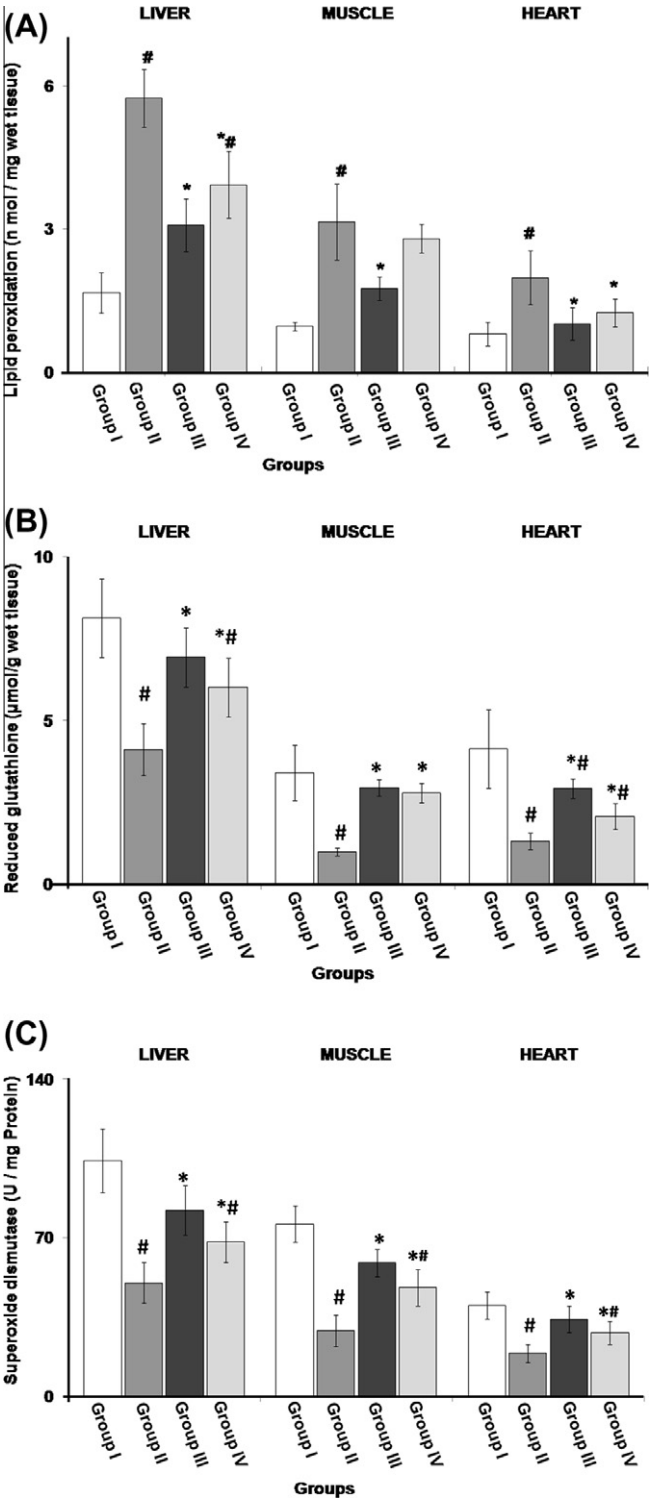

Fig. 2. Effect of 30 treatment of aqWC on (A) Lipid peroxidation, (B) Reduced Glutathione (C) Superoxide dismutase activity in liver, heart and muscle. Group I; healthy control, Group II; diabetic control, Group III; Diabetic + aqWC (250 mg/kg bw), Group IV; Diabetic + Glibenclamide (0.5 mg/kg bw). #$p < 0.05$ vs. Group I, *$p < 0.05$ vs. Group II, $n = 6$.

(GSH) is essential to maintain the structural and functional integrity of erythrocytes. During hyperglycemia, ROS bind to receptors that promote oxidative stress and generate intracellular oxidants (Hofmann et al., 1999). An increased polyol pathway flux during hyperglycemia is due to increase in aldose reductase (AR) activity in tissues which reduces glucose to sorbitol by consuming NADPH. Aldose reductase is being reported to metabolize GSH-lipid derived aldehyde adducts which results in decrease in GSH and subsequently increases oxidative stress (Srivastava et al., 1998; Bhatnagar and Srivastava, 1992). In DM, altered activities of these enzymes and reduced level of GSH have been observed which affect the ability to defend against oxidative stress (Maritim et al., 2003). The diabetic animals treated with aqWC showed significantly increased levels of GSH in blood and tissue homogenates. FRAP is a measure of the total antioxidant capacity, based on the reduction of ferrous ions by the effect of the reducing power of plasma constituents, and contributed by low molecular weight antioxidants of hydrophilic and/or hydrophobic nature. FRAP is said to give better biologically relevant information than provided by individual antioxidant measurements and may describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma (Benzie and Strain, 1999). Since in the diabetic state, FRAP activity has been decreased, whereas, it increased after aqWC treatment in DM may be due to modulatory effect of aqWC treatment.

5. Conclusions

It is evident from data that aqueous extract of fruit of *W.coagulans* (aqWC) possesses salutary effects on dyslipidemia and oxidative stress in diabetes mellitus. Moreover, it modulated the enzymes of lipid metabolism. However, studies to identify the active principle involved in these effects in diabetes mellitus are underway in our laboratory.

Conflict of Interest

The author(s) declare that they have no conflicts of interests.

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