

## ANTIDIABETIC EFFECT OF *SIDA CORDIFOLIA* (AQUEOUS EXTRACT) ON DIABETES INDUCED IN WISTAR RATS USING STREPTOZOTOCIN AND ITS PHYTOCHEMISTRY

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### ABSTRACT

Diabetes disorder has acquired an epidemic level and a threat for world population. All parts of plant *Sida cordifolia* are utilized for hypoglycemic activity. Bioactive component of aqueous extract of *Sida cordifolia* were phenolic, flavonoids, non-tannin and tannin. To create diabetes in Wistar rat, streptozotocin solution (0.5%) was freshly prepared in 0.1 M sodium citrate buffer (pH 4.5) and administered (55mg/kg) in overnight fasted rats intraperitoneally. Rats of group-A served as control and diabetes was induced in rats of group-B, C, D and E. Diabetic rats of group-B designated as diabetic control were treated with carboxy methyl cellulose. Whereas diabetic rats of groups-C and D were treated with aqueous extract of *Sida Cordifolia* at two dosages (200mg/kg and 400mg/kg respectively) by oral route after mixing with carboxy methyl cellulose for 28 days. Rats of group-E were treated with Glibenclamide (5mg/kg) mixed in carboxy methyl cellulose for 28 days. Higher dose (400mg/kg) of *Sida cordifolia* significantly increases ( $P<0.05$ ) body weight ( $186.66\pm 6.67g$ ) and significantly decreases blood glucose level ( $152.17\pm 16.92mg/dl$ ) of group-D diabetic rats on day 29th compared to day zero within the group. Also at same dose *Sida cordifolia* significantly ( $P<0.05$ ) decreases total cholesterol ( $55.12\pm 1.47mg/dl$ ), triglycerides ( $45.95\pm 1.56mg/dl$ ), LDL ( $24.12\pm 1.72mg/dl$ ), plasma creatinine ( $0.54\pm 0.07mg/dl$ ), plasma urea nitrogen ( $58.59\pm 3.25mmol/l$ ), lipid peroxidation ( $5.90\pm 0.34nmol$  MDA/ml) and significantly ( $P<0.05$ ) increases HDL ( $34.76\pm 1.66mg/dl$ ), catalase ( $59.98\pm 3.25Umol$  H<sub>2</sub>O<sub>2</sub>/min/mg of Hb) and superoxide dismutase ( $62.47\pm 2.33U/mg$  of Hb) activity of group-D diabetic rats on day 29th compared to day zero within the group. The bioactive component of plant mainly flavonoids and alkaloids produces anti-diabetic activity.

### INTRODUCTION

Diabetes in present scenario is the most common non-communicable disease worldwide. Due to its high prevalence, morbidity and mortality, diabetes is the third killer of mankind after cancer and cardiovascular disease [1]. Diabetes mellitus is one of the most common endocrine disorders that produces disturbances in insulin physiology leading to hyperglycemia [2]. In country like India diabetes is of great concern and is projected as "Diabetes Capital of World". It is classified into Type-I or insulin dependent diabetes mellitus (IDDM) and Type-II or non-insulin dependent diabetes mellitus (NIDDM) or insulin

deficiency [3]. Persistent hyperglycemia found in diabetic patient increases production of free radicals especially reactive oxygen species [4]. Reactive oxygen species (ROS) leads to cellular damage resulting in increased lipid peroxidation [5]. To combat the deteriorative effect of ROS, anti-oxidant enzymes viz., super oxide dismutase, catalase and glutathione peroxidase protects the cellular damage [6]. The effective control of blood glucose level is a key step in preventing diabetes [7]. Insulin is a drug of choice to control IDDM whereas oral hypoglycemic agents control NIDDM. However, prolonged therapy with conventional drugs leads to complications like blurred vision, hypoglycemia and deadly condition like coma [8]. To nullify these side effects, it is better to introduce herbs possessing hypoglycemic effect so that they can be used in combination with conventional available drugs. More than 800

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plants possess anti-diabetic activity. *Sida cordifolia* (Linn.) is a shrub of family Malvaceae, native of Brazil and commonly found throughout tropical and sub-tropical plains of India<sup>[9]</sup>. All parts of *Sida cordifolia* (roots, leaves, stem and seed) possesses activities like anti-rheumatic, antipyretic, diuretic, analgesic and hypoglycemic activity<sup>[10, 11]</sup>. Despite numerous measures that have been taken to control the diabetes, it is still a big challenge to combat emerging epidemic problem of diabetes. Therefore, the present study was conducted with the aim to evaluate phyto-chemistry and antidiabetic potential of aqueous extract of *Sida cordifolia* in streptozotocin induced diabetes in Wistar rats.

## MATERIALS AND METHODS

### 2.1. Extraction

Aerial parts of plant *Sida cordifolia* was collected from city Meerut (Uttar Pradesh, India). After scientific authentication, aerial part of plant was chopped into small pieces and kept for shade drying at room temperature (30-35°C). The dried part of plant was pulverized to powder and kept in thimble for aqueous extraction using Soxhlet apparatus.

### 2.2. Phytochemical Study:

Aqueous extract was subjected to quantitative estimation of phyto-chemicals such as total phenolic content<sup>[12]</sup>, total flavonoid content<sup>[13]</sup> and tannin<sup>[14]</sup> and non-tannin content<sup>[15]</sup>. Also

qualitative analysis was performed to analyse the presence of active constituents (alkaloids, glycosides, saponins, sterols, resins and flavonoids).

### 2.3. Experimental animals:

Study was conducted on healthy Wistar rats of either sex (170 to 230g) procured from Indian Institute of Integrative Medicine, Jammu (J&K), India. The animals were provided standard pelleted ration and *ad libitum* drinking tap water. A daily cycle of 12 h of light and 12h darkness period was provided to animals. Prior to start the experiment, rats were acclimatized to laboratory conditions for a period of about 3 weeks. The experimental protocol was duly approved by the Institutional Animal Ethics Committee.

### 2.4. Induction of diabetes:

Diabetic condition in rats was induced using 0.5% streptozotocin (STZ) solution in freshly prepared sodium citrate buffer (0.1 M, pH 4.5, 10mg/ml) and administered to overnight fasted Wistar rats (intra-peritoneally) at dose rate of 55mg/kg<sup>[16]</sup>. After STZ administration, rats were kept on 5% glucose solution made in drinking water for next 24h. Rats developed diabetes after 72 h of STZ administration and rats whose blood glucose level was found above 200 mg/dl<sup>[17]</sup> were recognized as diabetic rats. The blood glucose level was estimated by a glucometer (Accu-Check, Roche, Germany).

**Table.1. Showing Phyto-chemistry of aqueous extract of *Sida cordifolia* and its effect at 200 & 400mg/kg and Glibenclamide on blood glucose level after oral administration in diabetic Wistar rats (n=6)**

| Total Phenolic content<br>(mg of GAE/g of extract) | Total Flavonoids content<br>(mg Quercetin/g of extract) | Non-tannin content<br>(mg of GAE/g of extract) | Tannin content<br>(mg of GAE/g of extract) |
|--|---|--|--|
| 14.90 ± 1.16                                       | 551.60 ± 15.53  | 1.01 ± 0.41                                    | 11.53 ± 0.78                               |
| Treatment groups                                   | Blood Glucose level (mg/dl)                             |  |  |
|  | Day Zero  | Day 15 <sup>th</sup>                           | Day 29 <sup>th</sup>                       |
| Group-A (Normal control)                           | 111.67 ± 8.33 <sup>aA</sup>                             | 120.00 ± 7.30 <sup>aA</sup>                    | 118.33 ± 9.45 <sup>aA</sup>                |
| Group-B (Diabetic control)                         | 390.8 ± 16.62 <sup>bA</sup>                             | 408.00 ± 12.58 <sup>aD</sup>                   | 447.5 ± 20.75 <sup>bB</sup>                |
| Group-C ( <i>Sida cordifolia</i><br>@200mg/kg)     | 329.6 ± 23.16 <sup>cA</sup>                             | 321.6 ± 15.80 <sup>bA</sup>                    | 291.33 ± 21.96 <sup>bA</sup>               |
| Group-D ( <i>Sida cordifolia</i><br>@400mg/kg)     | 328.83 ± 32.53 <sup>bA</sup>                            | 240.33 ± 13.99 <sup>bB</sup>                   | 152.17 ± 16.92 <sup>aC</sup>               |
| Group-E<br>(Glibenclamide @5mg/kg)                 | 367.17 ± 38.01 <sup>bA</sup>                            | 215.00 ± 29.59 <sup>bB</sup>                   | 123.67 ± 12.73 <sup>aC</sup>               |

- Values are in Mean±SE, level of significance at 5% (P<0.05), and Similar superscript do not differ significantly
- Capital superscripts represent level of significance within the group,
- Small superscripts represent level of significance between the groups
- GAE (Gallic acid equivalent)

## 2.5. Experimental design:

A total of 30 healthy Wistar rats were divided into five groups (A, B, C, D & E) containing six animals in each. Diabetes was induced in rats of group-B, C, D and E whereas group-A served as control. Diabetic rats of group-B designated as diabetic control were treated only with carboxy methyl cellulose (1%). Whereas diabetic rats of group-C and D were treated with aqueous extract of *Sida cordifolia* orally at dosage of 200mg/kg and 400mg/kg mixed in carboxy methyl cellulose (1%) for 28 days, respectively. Diabetic rats of group-E were received glibenclamide (5mg/kg) orally for 28 days. Blood samples of about 1-2ml were collected from retro-orbital sinus of rats (day zero, 15<sup>th</sup> and 29<sup>th</sup>) using capillary tubes under inhalational anesthesia diethyl ether. Blood glucose level was measured at the time of its collection. Blood sample was centrifuged at 3000rpm for 15 min to harvest the plasma and kept in clean sterile glass test tubes at -20°C till further biochemical analysis. WBC buffy coat was removed from left over sediment and washed 2 to 3times with normal saline. Washed erythrocyte sediment was diluted with gentle pouring of normal saline solution in ratio of 1:1 (v/v) and thoroughly mixed to make 1% and 33% hemolysate. Finally 1% hemolysate was used to estimate anti-oxidant enzymes (catalase and superoxide dismutase) and 33% hemolysate for lipid peroxidation.

## 2.6. Biochemical, oxidative stress and body

## weight parameters estimation:

Biochemical indices such as blood glucose, triglycerides, cholesterol, high-density lipoproteins (HDL), plasma urea nitrogen and plasma creatinine were analyzed on day zero (pre-treatment) and 15<sup>th</sup> and 29<sup>th</sup> of post treatment using kits (Erba, HP, India). However, low-density lipoprotein (LDL) in plasma was estimated using the formula<sup>[18]</sup>.

$$\text{LDL (mg/dl)} = \text{TC} - \text{HDL} - \text{TG}/50$$

Where TC is total cholesterol, TG is total triglyceride

Weighing of rats at weekly interval was done to detect the effect of plant on body weight. Antioxidant enzymes viz. superoxide dismutase<sup>[19]</sup> and catalase<sup>[20, 21]</sup> and lipid peroxidation<sup>[22]</sup> were estimated using hemolysate.

## 2.7. Statistical analysis:

Data analysis was done using analysis of variance (ANOVA) in completely randomized design (CRD) at 5% level of significance (P<0.05). Level of significance (P<0.05) was tested using Duncan Multiple Range Test using SPSS programme<sup>[23]</sup>.

## RESULTS

### 3.1. Phytochemistry:

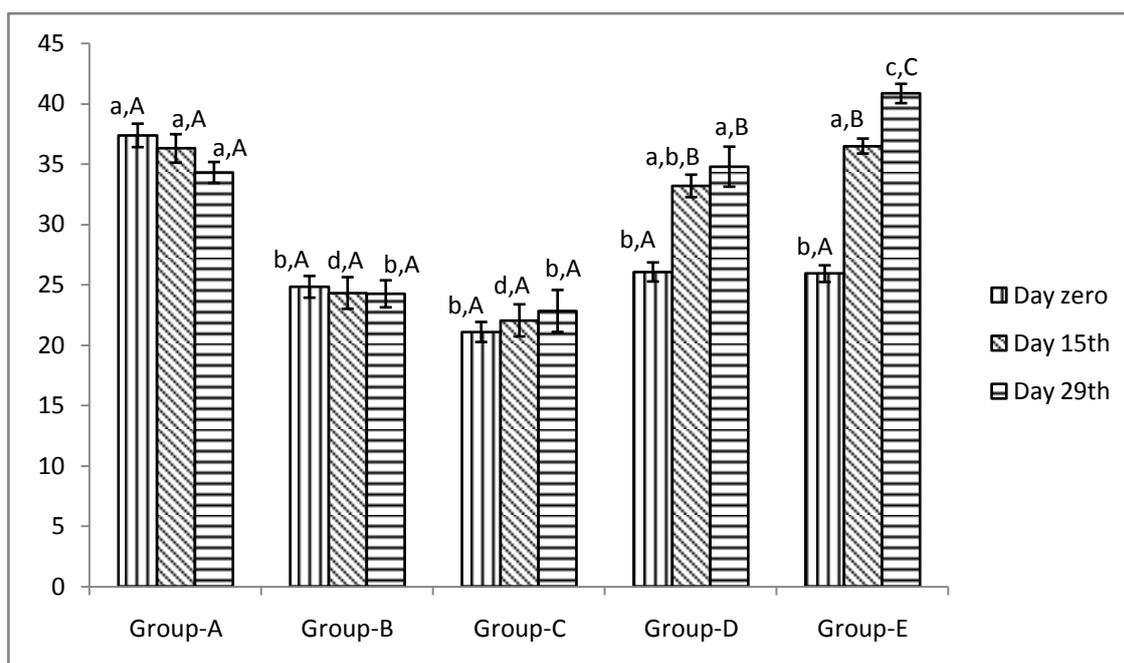
The percentage extractability of aqueous extract of *Sida cordifolia* was 10.52% (w/w) and

**Table.2. Showing effect of aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on Triglyceride and Cholesterol levels after oral administration in diabetic Wistar rats (n=6)**

| Treatment groups                            | Triglycerides (mg/dl)     |                          |                            |
|---|---------------------------|--------------------------|----------------------------|
|   | Day Zero                  | Day 15 <sup>th</sup>     | Day 29 <sup>th</sup>       |
| Group-A (Normal control)                    | 44.11±1.74 <sup>aA</sup>  | 43.57±1.02 <sup>aA</sup> | 42.81±1.59 <sup>abA</sup>  |
| Group-B (Diabetic control)                  | 84.52±1.22 <sup>bA</sup>  | 86.58±1.87 <sup>dA</sup> | 89.94±6.00 <sup>dA</sup>   |
| Group-C ( <i>Sida cordifolia</i> @200mg/kg) | 83.05±1.52 <sup>bA</sup>  | 82.59±1.87 <sup>cA</sup> | 81.72±1.20 <sup>cA</sup>   |
| Group-D ( <i>Sida cordifolia</i> @400mg/kg) | 84.25±1.90 <sup>bA</sup>  | 51.46±1.05 <sup>bb</sup> | 45.95±1.56 <sup>bc</sup>   |
| Group-E (Glibenclamide @5mg/kg)             | 86.37±1.71 <sup>bA</sup>  | 42.73±0.89 <sup>ab</sup> | 40.62±0.61 <sup>ac</sup>   |
|   | Cholesterol (mg/dl)       |                          |                            |
| Group-A (Normal control)                    | 51.36±2.09 <sup>aA</sup>  | 54.15±1.26 <sup>aA</sup> | 54.02±1.22 <sup>aA</sup>   |
| Group-B (Diabetic control)                  | 72.39±2.86 <sup>bcA</sup> | 77.95±0.81 <sup>dB</sup> | 80.74±1.00 <sup>dC</sup>   |
| Group-C ( <i>Sida cordifolia</i> @200mg/kg) | 71.25±2.51 <sup>bcA</sup> | 74.90±1.14 <sup>cA</sup> | 74.45 ±1.81 <sup>cA</sup>  |
| Group-D ( <i>Sida cordifolia</i> @400mg/kg) | 68.08±2.24 <sup>bA</sup>  | 60.96±2.68 <sup>bb</sup> | 55.12 ±1.47 <sup>aC</sup>  |
| Group-E (Glibenclamide @5mg/kg)             | 77.66±1.31 <sup>cA</sup>  | 54.46±1.21 <sup>ab</sup> | 51.96 ±1.30 <sup>abc</sup> |

- Values are in Mean±SE, level of significance at 5% (P<0.05), and Similar superscript do not differ significantly
- Capital superscripts represent level of significance within the group
- Small superscripts represent level of significance between the groups

**Figure 1: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on HDL levels after oral administration in Diabetic wistar rats (n=6)**



- Capital superscripts represent level of significance within the group at 5% ( $P < 0.05$ )
- Small superscripts represent level of significance between the groups at 5% ( $P < 0.05$ )

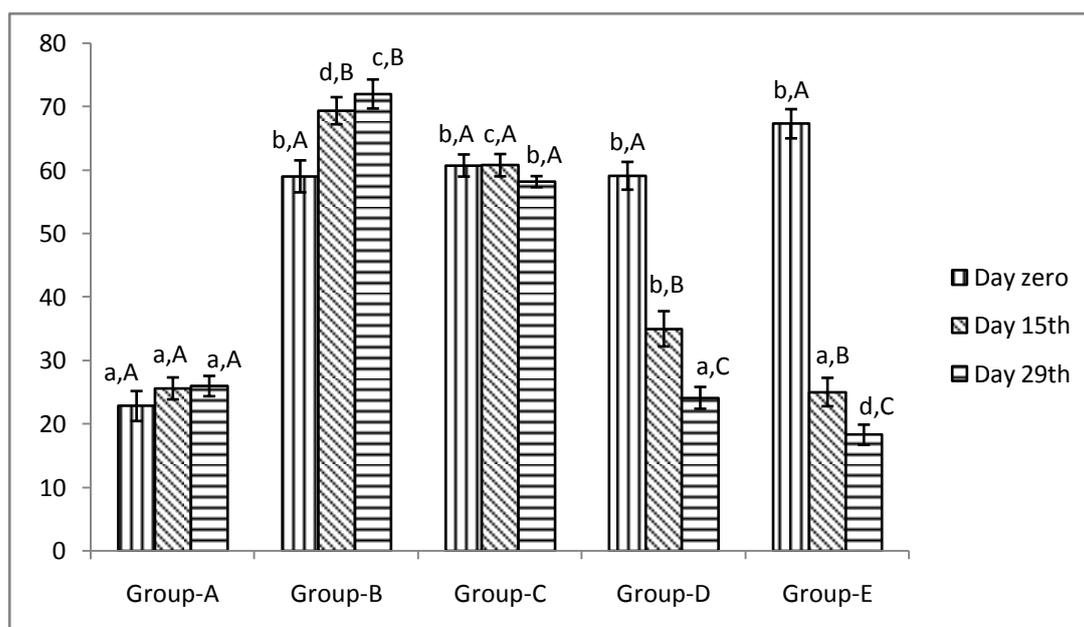
extract contain alkaloids, glycosides, saponins, sterols, resins, fixed oil and flavonoids. Quantitatively presence (mg/g) of phyto-chemicals in aqueous extract of *Sida cordifolia* was total flavanoids ( $551.60 \pm 15.53$ ), total phenolic content ( $14.90 \pm 1.16$ ), tannin content ( $11.53 \pm 0.78$ ) and

non-tannin content ( $1.01 \pm 0.41$ ) of (table.1).

### 3.2. Effect of *Sida cordifolia* on blood glucose and body weight of diabetic rats:

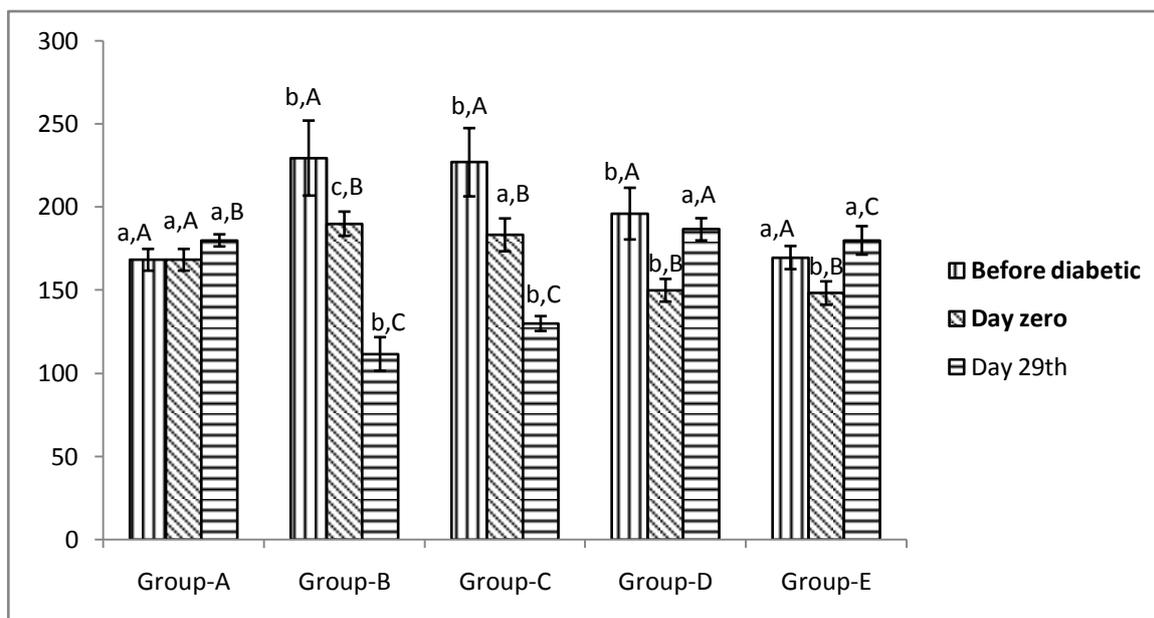
At day zero, a significant increase ( $P < 0.05$ ) of blood glucose level (table.1) was found in diabetic

**Figure 2: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on LDL levels after oral administration in Diabetic wistar rats (n=6)**



- Capital superscripts represent level of significance within the group at 5% ( $P < 0.05$ )
- Small superscripts represent level of significance between the groups at 5% ( $P < 0.05$ )

**Figure 3: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on Body weight after oral administration in Diabetic wistar rats (n=6)**

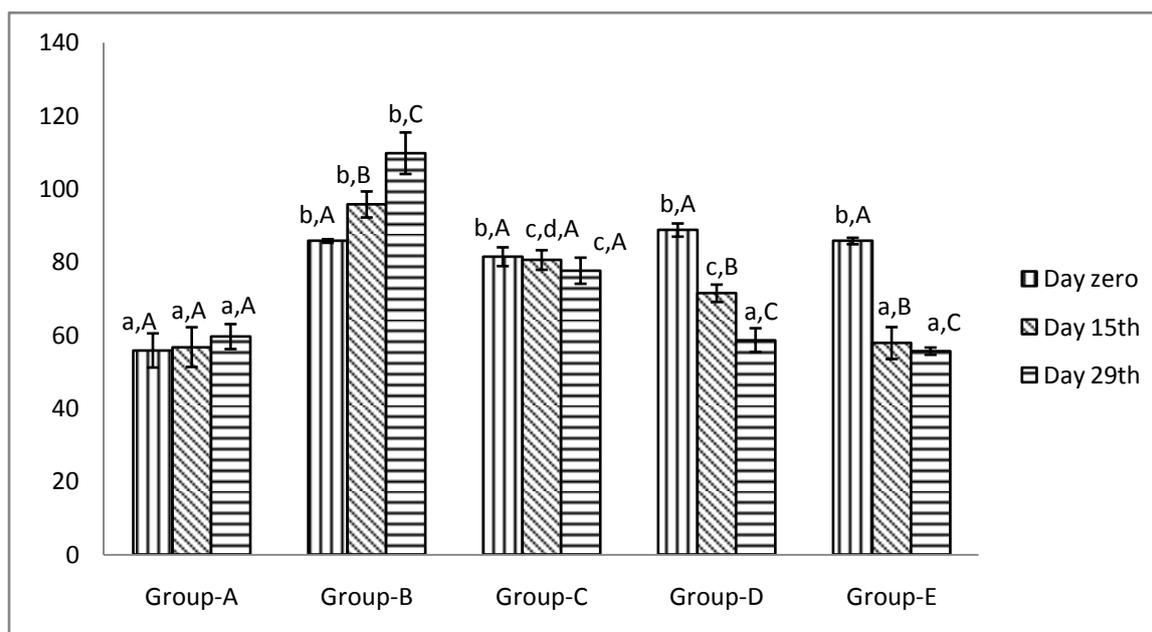


- Capital superscripts represent level of significance within the group at 5% ( $P < 0.05$ )
- Small superscripts represent level of significance between the groups at 5% ( $P < 0.05$ )

rats of group-B ( $390.8 \pm 16.62g$ ), group-C ( $329.6 \pm 23.16g$ ), group-D ( $328.83 \pm 32.53g$ ), and group-E ( $367.17 \pm 38.01g$ ) as compared to control rats of group-A ( $111.67 \pm 8.33g$ ). At dosage 200mg/kg (*Sida cordifolia*), a non-significant reduction of blood glucose level was found on day

15<sup>th</sup> ( $321.6 \pm 15.80g$ ) and 29<sup>th</sup> ( $291.33 \pm 21.96g$ ) as compared to day zero ( $329.6 \pm 23.16g$ ) within diabetic rats of group-C. However at higher dosage of 400mg/kg (*Sida cordifolia*), a significant decrease ( $P < 0.05$ ) of blood glucose level was found within diabetic rats of group-D on day 15<sup>th</sup>

**Figure 4: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on Plasma urea nitrogen levels after oral administration in Diabetic wistar rats (n=6)**



- Capital superscripts represent level of significance within the group at 5% ( $P < 0.05$ )
- Small superscripts represent level of significance between the groups at 5% ( $P < 0.05$ )

(240.33±13.99g) and 29<sup>th</sup> (152.17±16.92g) as compared to day zero (328.83±32.53g) and also compared to diabetic control rats of group-B (447.5±20.75g). In group-D diabetic rats, decreased blood glucose level on day 29<sup>th</sup> (152.17±16.92g) was comparable to group-A control rats (118.33±9.45g). Similar significant (P<0.05) decrease of blood glucose level was also found in glibenclamide treated diabetic rats of group-E (123.67±12.75g).

A significant decrease (P<0.05) of body weight (fig.3) was found in diabetic rats of group-B on day zero (190 ±7.30g) and 29<sup>th</sup> (111.67±10.13g) as compared to rats before diabetes within same group (229.49±22.56g). However in *Sida cordifolia* (400mg/kg) and glibenclamide treated diabetic rats of groups-D and group-E, a significant increase (P<0.05) of body weight was found within same group (fig.3) on day 29<sup>th</sup> (186.66±6.67 and 180±8.56g respectively) as compared to day zero (150±6.83 and 148.33±7.03g respectively). Whereas *Sida cordifolia* at 200mg/kg was not produced any significant change in body weight on day 29<sup>th</sup> (130±14.47g) compared to day zero (183.33±9.88g) within diabetic rats of group-C.

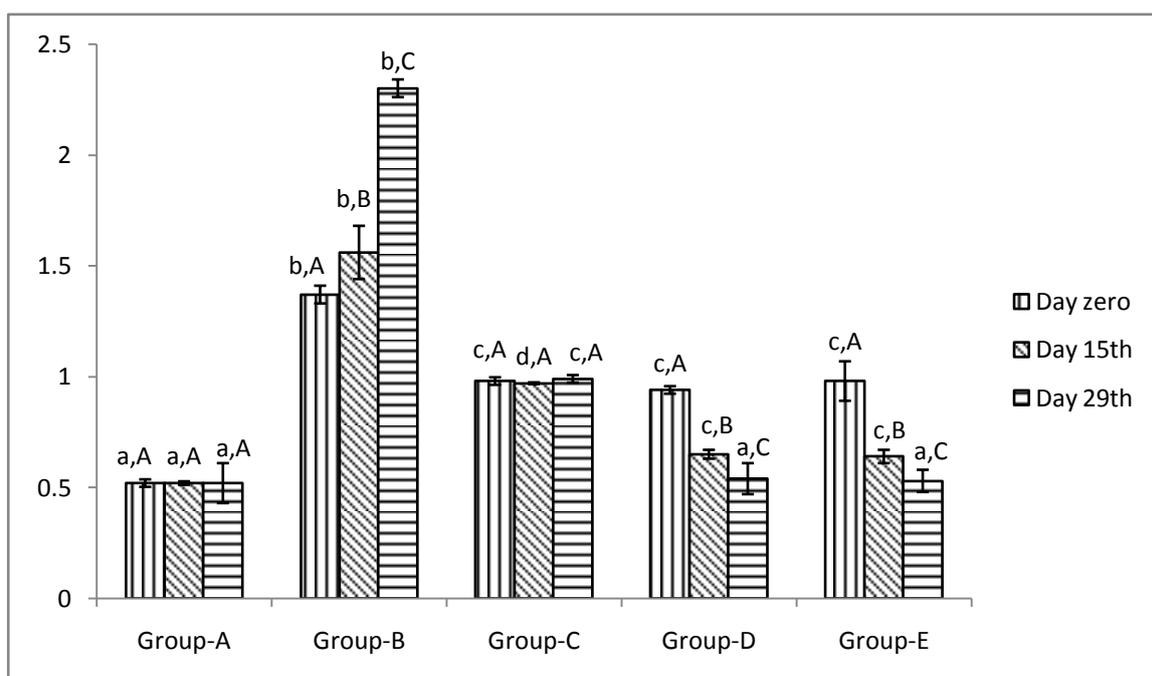
### 3.3. Effect of *Sida cordifolia* on triglyceride, cholesterol, HDL and LDL levels of diabetic rats:

A significant increase (P<0.05) of triglycerides level (table.2) was found in diabetic rats of group-

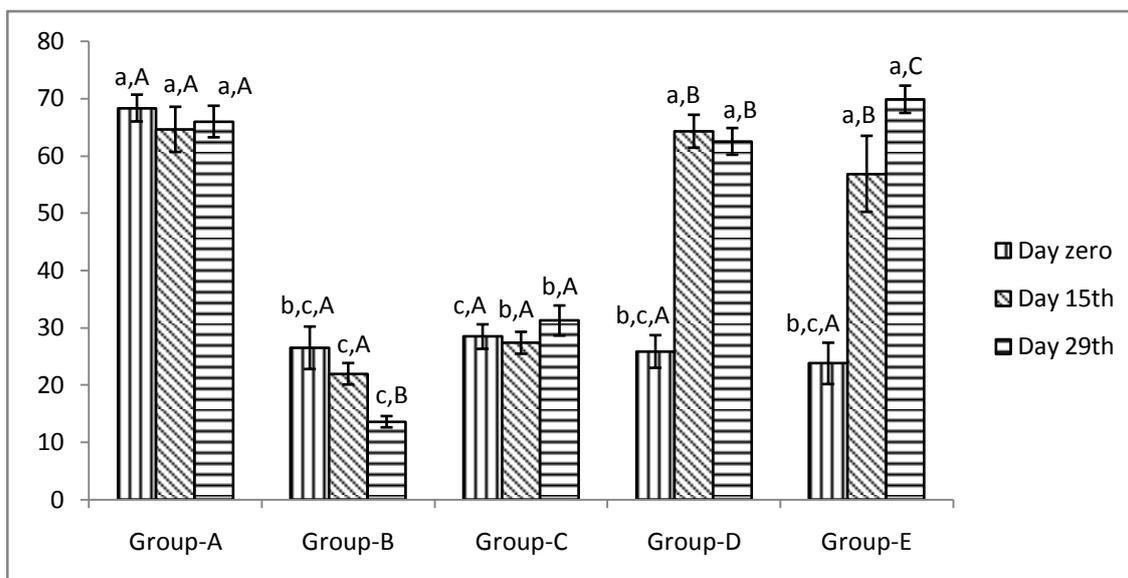
B (84.52±1.22mg/dl), group-C (83.05±1.52 mg/dl), group-D (84.25±1.90 mg/dl) and group-E (86.37±1.71 mg/dl) on day zero compared to control rats of group-A. However in *Sida cordifolia* (400mg/kg) and glibenclamide (5mg/kg) treated group-D and group-E rats, a significant decrease (P<0.05) of triglyceride level was found on day 15<sup>th</sup> (51.46±1.05 and 42.73±0.89, respectively) and 29<sup>th</sup> (45.95±1.56 mg/dl and 40.62±0.61 mg/dl, respectively) as compared to day zero within same group and also compared to diabetic control rats of group-B, respectively. On day 29<sup>th</sup>, a non-significant change of triglyceride level was found in *Sida cordifolia* and glibenclamide treated group-D (45.95±1.56 mg/dl) and group-E (40.62±0.61 mg/dl) rats, respectively as compared to control rats of group-A (42.81±1.59 mg/dl). Although 200mg/kg dose of *Sida cordifolia* was not sufficient to produce significant change in triglyceride level on days 15<sup>th</sup> (82.59±1.87 mg/dl) and 29<sup>th</sup> (81.72±1.20 mg/dl) as compared to day zero (83.05±1.52 mg/dl) within diabetic rats of group-C.

A significant increase (P<0.05) of cholesterol level (table.2) was found on day zero in diabetic rats of group-B (72.39±2.86 mg/dl), group-C (71.25±2.51 mg/dl), group-D (68.08±2.24 mg/dl) and group-E (77.66±1.31 mg/dl) as compared to group-A rats (51.36±2.09 mg/dl). *Sida cordifolia* (400mg/kg) and glibenclamide (5mg/kg) were produces a significant decrease (P<0.05) of cholesterol level in group-D and group-E rats on

**Figure 5: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on plasma creatinine levels after oral administration in Diabetic wistar rats (n=6)**



- Capital superscripts represent level of significance within the group at 5% (P<0.05)
- Small superscripts represent level of significance between the groups at 5% (P<0.05)

**Figure 6: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on SOD levels after oral administration in Diabetic wistar rats (n=6)**

- Capital superscripts represent level of significance within the group at 5% ( $P < 0.05$ )
- Small superscripts represent level of significance between the groups at 5% ( $P < 0.05$ )

day 15<sup>th</sup> ( $60.96 \pm 2.68$  mg/dl and  $54.46 \pm 1.21$  mg/dl, respectively) and 29<sup>th</sup> ( $55.12 \pm 1.47$  mg/dl and  $51.96 \pm 1.30$  mg/dl, respectively) as compared to day zero within same group and also compared to diabetic rats of group-B, respectively. However, a non-significant change of cholesterol level was found in rats of group-C on day 15<sup>th</sup> ( $74.90 \pm 1.14$  mg/dl) and 29<sup>th</sup> ( $74.45 \pm 1.81$  mg/dl) as compared to day zero within same group ( $71.25 \pm 2.51$  mg/dl).

On day zero, a significant increase ( $P < 0.05$ ) of LDL level (fig.2) was found in diabetic rats of group-B ( $59.02 \pm 2.52$  mg/dl), group-C ( $60.73 \pm 1.74$  mg/dl), group-D ( $59.11 \pm 2.19$  mg/dl) and group-E ( $67.31 \pm 2.30$  mg/dl) as compared to group-A rats ( $22.83 \pm 2.36$ ). *Sida cordifolia* (400mg/kg) and glibenclamide (5mg/kg) treatment significantly decreases ( $P < 0.05$ ) LDL level in rats of group-D and group-E on day 15<sup>th</sup> ( $35.0 \pm 2.78$  mg/dl and  $25.04 \pm 224$  mg/dl, respectively) and day 29<sup>th</sup> ( $24.12 \pm 1.72$  mg/dl and  $18.31 \pm 1.59$  mg/dl, respectively) as compared to day zero within same group and also as compared to diabetic rats of group-B, respectively. A non-significant change in LDL level was found in group-C on day 15<sup>th</sup> ( $60.78 \pm 1.76$  mg/dl) and day 29<sup>th</sup> ( $58.19 \pm 0.87$  mg/dl) as compared to day zero within same group.

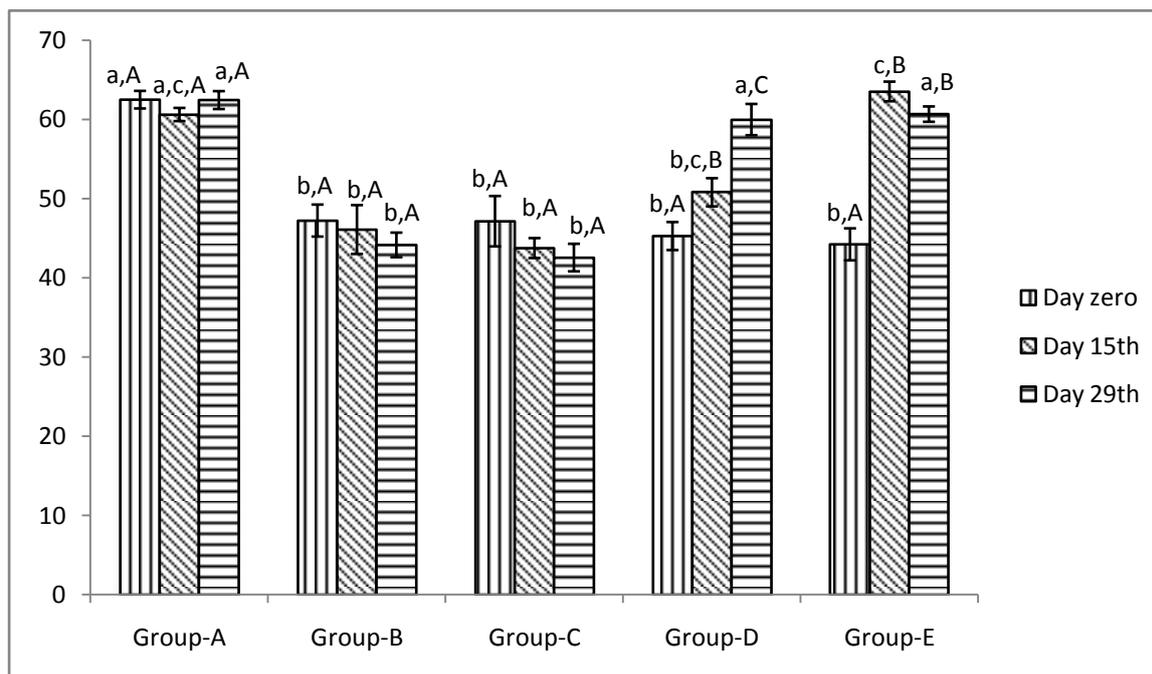
A significant decrease ( $P < 0.05$ ) of HDL level (fig.1) was found in diabetic rats of group-B ( $24.81 \pm 0.9$ ) on day zero as compared to group-A rats ( $37.35 \pm 0.97$  mg/dl). However, *Sida cordifolia* (400mg/kg) and glibenclamide treated diabetic

rats of groups-D and E, a significant increase ( $P < 0.05$ ) of HDL level was found on day 15<sup>th</sup> ( $33.17 \pm 0.93$  mg/dl and  $36.47 \pm 0.62$  mg/dl, respectively) and 29<sup>th</sup> ( $34.76 \pm 1.66$  mg/dl and  $40.82 \pm 0.8$  mg/dl, respectively) as compared to day zero within same group ( $26.04 \pm 0.79$  mg/dl and  $25.90 \pm 0.69$  mg/dl, respectively). On day 29<sup>th</sup>, HDL level of group-D and E was similar to control rats of group-A ( $34.28 \pm 0.87$  mg/dl). Although *Sida cordifolia* at 200mg/kg was not sufficient to decrease the HDL level of diabetic rats of group-C to a significant level on day 15<sup>th</sup> ( $22.03 \pm 1.33$  mg/dl) and 29<sup>th</sup> ( $22.81 \pm 1.74$  mg/dl) as compared to day zero ( $21.07 \pm 0.83$  mg/dl) within same group.

#### 3.4. Effect of *Sida cordifolia* on plasma urea nitrogen and plasma creatinine level of diabetic rats:

A significant increase ( $P < 0.05$ ) of plasma urea nitrogen (fig.4) and plasma creatinine (fig.5) level were found on day zero in group-B ( $85.68 \pm 0.48$  mmol/l and  $1.37 \pm 0.01$  mg/dl, respectively), group-C ( $81.39 \pm 2.57$  mmol/l and  $0.98 \pm 0.02$  mg/dl, respectively), group-D ( $88.67 \pm 1.8$  mmol/l and  $0.94 \pm 0.01$  mg/dl, respectively) and group-E rats ( $85.68 \pm 0.89$  mmol/l and  $0.98 \pm 0.08$  mg/dl, respectively) as compared to control rats of group-A ( $55.76 \pm 4.67$  mmol/l and  $0.52 \pm 0.01$  mg/dl, respectively). Although a significant decrease ( $P < 0.05$ ) of plasma urea nitrogen and plasma creatinine level were observed in *Sida cordifolia* (400mg/kg) and glibenclamide (5mg/kg) treated diabetic rats of group-D ( $58.59 \pm 3.25$  mmol/l and

**Figure 7: Showing the effect aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on Catalase level after oral administration in Diabetic wistar rats (n=6)**



- Capital superscripts represent level of significance within the group at 5% ( $P < 0.05$ )
- Small superscripts represent level of significance between the groups at 5% ( $P < 0.05$ )

0.54±0.07 mg/dl, respectively) and group-E (55.57±0.99 mmol/l and 0.53±0.05 mg/dl, respectively) on day 29<sup>th</sup> as compared to day zero within same group and as compared to diabetic rats of group-B, respectively. On day 29<sup>th</sup> Plasma urea nitrogen and plasma creatinine level in treated rats of groups-D and E was similar to control rats of group-A (59.56±3.41 mmol/l and 0.52±0.09 mg/dl, respectively). However, at 200mg/kg dose of *Sida cordifolia* was not enough to reduce plasma urea nitrogen and plasma creatinine level significantly in diabetic rats of group-C.

### 3.5. Effect of *Sida cordifolia* on antioxidant enzymatic activity (Catalase and SOD) and on Lipid peroxidation of diabetic rats:

A significant decrease ( $P < 0.05$ ) of SOD (fig.6) and Catalase (fig.7) activity were found on day zero in diabetic rats of group-B (26.47±3.71U/mg of Hb and 47.23±2.02 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively) as compared to control rats of group-A (68.28±2.34 U/mg of Hb and 62.49±1.11 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively). However *Sida cordifolia* (400mg/kg) and glibenclamide treated diabetic rats of groups-D and E, a significant increase ( $P < 0.05$ ) of SOD and catalase activity were found on day 29<sup>th</sup> in group-D (62.47±2.33 U/mg of Hb and 59.98±1.97 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively) and group-E (69.81±2.38 U/mg of

Hb and 60.67±0.97 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively) as compared to day zero within same group and as compared rats of group-B, respectively (13.58±0.92 U/mg of Hb and 44.16±1.55 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively). On day 29<sup>th</sup> catalase and SOD activity in group-D and E rats were similar to level found in control rats of group-A (62.44±1.13 U/mg of Hb and 65.93±2.75 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively). Although *Sida cordifolia* at 200mg/kg, dose was not sufficient enough to decrease catalase and SOD activity to significant level in diabetic rats of group-C on day 29<sup>th</sup> (42.56±1.74 U/mg of Hb and 31.23±2.62 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively) as compared to day zero (47.15±3.18 U/mg of Hb and 28.42±2.14 Umol H<sub>2</sub>O<sub>2</sub>/min/mg of Hb, respectively).

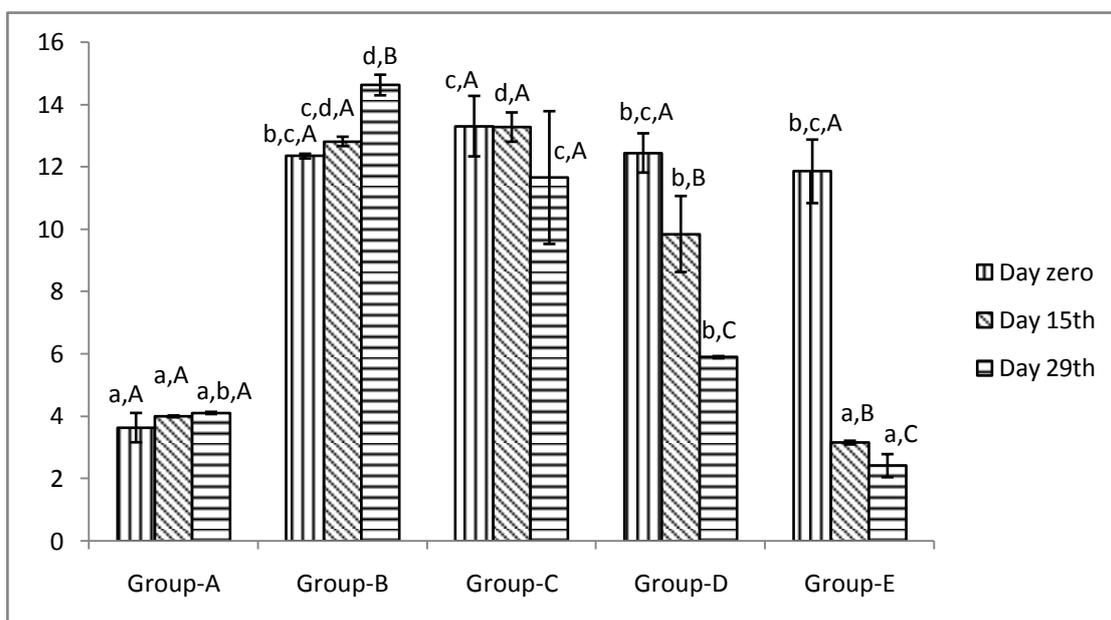
On day zero (fig.8) a significant increase ( $P < 0.05$ ) of lipid peroxidation was found in group-B (12.35±0.07nmol MDA/ml), group-C (13.31±0.97nmol MDA/ml), group-D (12.45±0.63nmol MDA/ml) and group-E rats (11.86±1.01nmol MDA/ml) as compared to control rats of group-A (3.64 ±0.47nmol MDA/ml). However *Sida cordifolia* (400mg/kg) and glibenclamide (5mg/kg) treated rats of group-D and group-E, a significant decrease ( $P < 0.05$ ) of lipid peroxidation was found on day 15<sup>th</sup> (9.85±1.22nmol MDA/ml and 3.16±0.05nmol MDA/ml) and 29<sup>th</sup> (5.90±0.34nmol MDA/ml and

2.42±0.37nmol MDA/ml) as compared to day zero within same group and compared to diabetic control rats of group-B (12.82 ±0.15 and 14.63 ±0.33 nmol MDA/ml), respectively. Lipid peroxidation in treated groups-D and group-E on day 29<sup>th</sup> were comparable to control rats of group-A (4.11±0.03nmol MDA/ml). Although dosage 200mg/kg of *Sida cordifolia* on day 15<sup>th</sup> (13.28±0.47nmol MDA/ml) and 29<sup>th</sup> (11.66±2.13nmol MDA/ml) was not sufficient to produce significant change of lipid peroxidation in diabetic rats of group-C as compared to day zero.

2011<sup>[9]</sup>, Pawar et al. 2011<sup>[24]</sup>, and Ahmad et al. 2014b<sup>[16]</sup>.

Diabetes is characterized with loss of body weight as body protein or fats are being utilized for energy generation through gluconeogenesis<sup>[25]</sup>. Aqueous extract of *Sida cordifolia* at 400mg/kg significantly improved the (P<0.05) body weight of diabetic rats (186.66±6.67g), indicates the possible role of *Sida cordifolia* extract in restoration of protein metabolism and supported by Kaur et al. 2011<sup>[9]</sup>. Ameliorative effect of plant to restore the body weight of diabetic rats may be through

**Figure 8: Showing the effect Aqueous extract of *Sida cordifolia* (200 & 400mg/kg) and Glibenclamide on LPO level after oral administration in Diabetic Wistar-rats (n=6).**



- Capital superscripts represent level of significance within the group at 5% (P<0.05)
- Small superscripts represent level of significance between the groups at 5% (P<0.05)

## DISCUSSION

Diabetes is a common metabolic disorder usually associated with deadly diseases viz. arteriosclerosis, nephritis and hypertension. *Sida cordifolia* possesses hypoglycemic activity<sup>[9]</sup> and its hypoglycemic potency depends upon the parts of plant, dosage and types of extract (aqueous, alcoholic, ethanolic). Therefore, present study was programmed with the aim to evaluate antidiabetic potential of aqueous extract of aerial part of plant (*Sida cordifolia*).

Phyto-chemistry or bioactive components of plants are needed to produce therapeutic property. Aqueous extract of *Sida cordifolia* revealed the presence of reducing sugars, glycosides, resins, alkaloids, flavonoids, saponins and sterols supported by findings of Kaur et al.

increasing glucose metabolism or by reversal of gluconeogenesis<sup>[26]</sup>.

Streptozotocin is toxic to pancreatic beta cells and thus widely used to induce the Diabetes Mellitus. Aqueous extract of *Sida cordifolia* at 400mg/kg significantly reduces blood (P<0.05) glucose level within a treatment period of 28 days (152.17±16.92mg/dl), indicating hypoglycemic activity of the plant. Similarly, Kanth and diwan 1999<sup>[10]</sup>, Kaur et al. 2011<sup>[9]</sup> and Ahmad et al. 2014b<sup>[16]</sup> also reported hypoglycemic potential of *Sida cordifolia* and said hypoglycemic effect of the plant may be through an increase of insulin release by stimulating pancreatic beta cells. Hypoglycemic activity of *Sida cordifolia* may also be by increasing glucose metabolism<sup>[9]</sup>. Bioactive component alkaloids and flavonoids also participate to increase insulin secretion and peripheral glucose utilization<sup>[27]</sup>. In present study higher

concentration of flavonoids were found in aqueous extract of *Sida cordifolia*.

Diabetes also influences fat metabolism indicated by increase of total cholesterol, triglycerides and LDL and decrease in HDL levels, resulting coronary artery diseases [28]. In present study significant rise ( $P < 0.05$ ) of total cholesterol, triglycerides and LDL and decrease in HDL were found in STZ induced diabetic rats. Aqueous extract of *Sida cordifolia* at 400 mg/kg for 28 days significantly decreases ( $P < 0.05$ ) total cholesterol ( $55.12 \pm 1.47$  mg/dl), triglycerides ( $45.95 \pm 1.56$  mg/dl) and LDL ( $24.12 \pm 1.72$  mg/dl) and significantly increases HDL ( $34.76 \pm 1.66$  mg/dl) level of diabetic rats. Similar to present study **Kaur et al.** 2011 [9] also supported present finding at 1000mg/kg (oral). Hypocholesteremic effect of plant may through an overall inhibition of fatty acid synthesis [9, 29]. *Sida cordifolia* significantly reduces LDL levels of diabetic rats through activating LDL receptors in hepatocyte or by inhibiting cholesterol synthesis pathway [30]. *Sida cordifolia* treated group decreases triglyceride level by increasing insulin levels or may activate lipoprotein lipase which hydrolyzes triglycerides [9].

Diabetic patients are at higher risk of developing nephropathy. Presence of higher amounts of glucose in blood makes kidney to work more. Thus in diabetes increase of serum creatinine and BUN level were found [31]. *Sida cordifolia* at 400mg/kg significantly reduces ( $P < 0.05$ ) the plasma creatinine ( $0.54 \pm 0.07$  mg/dl) and plasma urea nitrogen ( $58.59 \pm 3.25$  mmol/l) of diabetic rats. Available scientific literature showed that *Sida cordifolia* produces nephroprotective activity either through its antioxidant potential [32] or by correcting blood glucose level [9] or by both pathways [16]. Present finding also supported through an increase in antioxidant enzymatic activity (SOD, CAT) and decrease in lipid peroxidation indicating *Sida cordifolia* prevent oxidative damage of diabetic rats.

Diabetic Mellitus generate reactive oxygen species leading to oxidative damage [33]. Malondealdehyde (MDA) is the terminal product of lipid peroxidation, thus concentration of MDA determines the extent of oxidative damage produced in diabetes rats [33, 34]. Oxidative stress decreases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase [33, 35, 36, 37]. Enzymes superoxide dismutase and catalase [37] participates in alleviating free radicals generated oxidative damage in diabetes. *Sida cordifolia* at 400mg/kg showed a significant increase ( $P < 0.05$ ) of catalase ( $59.98 \pm 1.97$  UmolH<sub>2</sub>O<sub>2</sub>/min/mg of Hb) and SOD ( $62.47 \pm 2.33$  U/mg of Hb) and also significant

decrease ( $P < 0.05$ ) of lipid peroxidation ( $5.90 \pm 0.34$  nmol MDA/ml) in STZ induced diabetes in Wistar rats within treatment period of 28 days. However at lower dose 200mg/kg of *Sida cordifolia*, no such significant change was found. Similar, antioxidant property of *Sida cordifolia* was reported by **Dhalwal et al.** 2005 [38] and **Pawar et al.** 2011 [24] through scavenging of the free radicals. **Auddy et al.** 2003 [39] also reported *in vivo* and *in vitro* antioxidant activity of whole plant (*Sida cordifolia*). Hence, aqueous extract of *Sida cordifolia* at dosage 400mg/kg is sufficient to alleviate the lipid peroxidation and also increases the antioxidant enzymatic (SOD, CAT) activities through free radicals scavenging. Aqueous extract of *Sida cordifolia* contain bioactive component viz. phenolic, flavonoids, non-tannin and tannin. Amongst the bioactive component, highest concentration of flavonoids were found and thought to be the chief ingredient helping to produce anti-diabetic activity.

*Sida cordifolia* is considered safe as far as its toxicity potential is concerned. As per the literature its LD50 is more than 3g/kg [40], thus *Sida cordifolia* can be used safely in medicinal practices without causing toxicity.

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