Chapter 4: RESULTS
The study was aimed to analyze the ability of crude hydro-ethanolic extracts of *R. communis* to manage OS in alloxan induced, diabetic, adult, male swiss albino mice. Initially, the various parts of the plant were collected, processed and soxhlet extracted in hydro-ethanol (50% ethanol). The plant extracts had been screened for estimating their phyto-chemical nature followed by *in vitro* analysis of their anti-diabetic and antioxidant capacity using various biochemical assays viz. α-glucosidase, α-amylase, ABTS, DPPH, Metal Ion scavenging, SOD, CAT, GPx. With an *in vitro* overview of the possible anti-diabetic and antioxidative abilities of various extracts, they were further used for *in vivo* evaluation, which was done by treating the Alloxan induced diabetic swiss albino, male mice with the plant’s crude extracts for a period of 45 days. After the experimental period, their hepatic, renal and pancreatic tissues were checked for the amount oxidative stress which was compared with normal, diabetic and standard drug treated diabetic mice. Following this a comparative analysis of genetic expression of enzymatic antioxidant genes was also carried out among the organs of normal, diabetic, standard drug treated and the most efficient crude extract treated groups. A pure compound was then isolated from the most efficient crude extract, characterized and was further tested for evaluation of its anti-diabetic and antioxidative efficiency.

4.1 Evaluation of phytochemical constituents and *in vitro* anti-diabetic and antioxidative potential of hydroethanloic extracts of *R. communis*.

4.1.1. Phytochemical Screening

The hydroethanolic (50% ethanolic) extracts of various parts of *R. communis* were tested for the presence of some bioactive constituents including alkaloids, fatty acids, saponins, resins, phenols, steroids, terpenoids, glycosides, tannins, flavanoids to further test their medicinal potency.

Table 4.1 exhibits the presence of many phytochemical constituents in the various extracts of *R. communis*. The RCS however, showed the presence of highest number of phyto-constituents studied namely, Glycosides (+++), Terpenoids(+++), Phenols(+++) in high amount; Saponins(+), Steroids(+), Tannins(++) in a moderate amount; Flavonoids(+) and Alkaloids(+) in low amounts.
The RCR exhibited the presence of Glycosides (+++) in ample, moderate Alkaloids (++), Flavonoids (++), Phenols (++), Steroids (++), and Tannins (+++) and low Saponins(+).

The RCL displayed the presence of a high amount of Flavonoids(+++), moderate amount of Alkaloids(++), Saponins(++), and steroids(++) whereas, Glycosides(+) and Phenols(+) showed their presence in low amounts.

Fatty acids were found absent in all the extracts except for the RCP which showcased an altogether different pattern of the presence of high amount of Fatty acids(+++). Besides, Alkaloids(++), Flavonoids(++), Glycosides(++), Terpenoids(++) were present moderately. While, Resins(+) and Saponins(+) were also present in the RCP but in low amount. However, Phenols, Steroids and Tannins were absent.

Alkaloids(+), Flavonoids(++), Glycosides(++), Phenols(++), Saponins(+), Steroids(+), Tannins(+), Tannins(++) and Terpenoids(+) were found to be present in the RCF.

### 4.1.2 In vitro anti-diabetic potential

In order to curb diabetes the extracts under study must limit the glucose production in the body so as to control hyperglycemia. The potency of extracts to be possible anti-diabetic agent was estimated by measuring α-glucosidase and α-amylase inhibition.

#### 4.1.2.1 α-glucosidase inhibiting ability of various extracts

All concentrations of the extracts showed some amount of α-glucosidase inhibition according to the results displayed in Table 4.2. The estimated IC\(_{50}\) values of RCL, RCF, RCS and RCR were 348 μg/ml, 617 μg/ml, 624 μg/ml and 598 μg/ml respectively. The RCP show highest IC\(_{50}\) out of all, being 988 μg/ml. However, the IC\(_{50}\) values for control Acarbose was 230.71 μg/ml.

The α-glucosidase inhibition by RCL was found to be higher than that of Acarbose at 600, 800 and 1000 μg/ml. Similarly the α-glucosidase inhibition of RCR was also observed to be higher than that of Acarbose at 800 and 1000 μg/ml.

Thus, it could be concluded that the α-glucosidase inhibition activities of RCL and RCR were somewhat comparable to that of the standard Acarbose. Also, lower IC\(_{50}\) values of RCL and RCR revealed them as antidiabetic with greater potential as compared to other
extracts, whereas, RCP was found to have the least anti-diabetic ability amongst all the five extracts studied.

4.1.2.2 α-amylase inhibiting ability of various extracts

Table 4.2 displayed that extracts at all concentrations show some amount of α-amylase inhibition. The IC$_{50}$ values calculated for the various extracts viz., RCL, RCF, RCS and RCR are 320 μg/ml, 602 μg/ml, 668μg/ml and 401μg/ml respectively. The RCP however exhibited an IC$_{50}$ of 805μg/ml which was the highest out of all the extracts studied. The control used, Acarbose had an IC$_{50}$ values of 325.50 μg/ml. RCL caused α-amylase inhibition which is found to be higher than that of Acarbose at all concentrations. This was followed by RCR which displayed inhibition % higher than that of Acarbose at 800 and 1000 μg/ml. Thus, it could be deduced that the α-amylase inhibition activities of RCL were higher than that of Acarbose and those of RCR were somewhat comparable to that of the standard. Also, lowest IC$_{50}$ values of RCL and RCR indicated them as anti-diabetic with greater potential as compared to other extracts. RCP emerged out with lowest anti-diabetic potential amongst all.

4.1.3 In vitro anti-oxidative potential

In order to treat diseases medicinal plants have to be efficient chemo-protectors which can protect the body from various reactive species and thus keep the OS under control. The anti-oxidative nature of plant extracts were analyzed in vitro by means of various Free radical scavenging assays(4.1.3.1-4.1.3.3) and estimating activities of various antioxidant enzymes (4.1.3.4-4.1.3.6)

4.1.3.1 ABTS scavenging

Table 4.3 summarized the ABTS radical scavenging capacities of all the extracts of _R. communis_ as % inhibition of ABTS$^+$ radicals.

The standard used, Ascorbic acid depicted an IC$_{50}$ value of 163.3 μg/ml.
RCL displayed the highest capacity to scavenge ABTS$^+$ at 800 and 1000 μg/ml concentration, which were 160.33 ±4.818 % and 224.98± 6.213% respectively, with an IC$_{50}$ value of 185.2 μg/ml which was the lowest amongst all the five extracts.

RCR exhibited the second best scavenging after leaves. Its inhibition % at 800 and 1000 μg/ml were calculated as 162.15±2.022 and 206.59± 2.7 respectively and its IC$_{50}$ value being 197.4

RCS and RCF showed almost similar scavenging patterns lower than that of the standard. Their IC$_{50}$ values being 230.7 μg/ml and 292.8 μg/ml

While RCP saw poor scavenging ability as its inhibition percentages were significantly very less than that of Ascorbic acid standard, while its IC$_{50}$ was calculated to be highest, 802 μg/ml.

From the results it is evident that the plant’s leaf and root extract showed most efficient inhibition which was comparable to that of standard Ascorbic acid.

**4.1.3.2 DPPH scavenging**

The percentage inhibition of DPPH by various hydro-ethanolic extracts of *R. communis* was recorded in Table 4.3. According to the results obtained, it could be claimed that all the extracts exhibit brilliant DPPH scavenging ability by displaying higher inhibition percentages and lower IC$_{50}$ values as compared to the standard EDTA, whose IC$_{50}$, 625 μg/ml was recorded to be the highest after RCP with an IC$_{50}$ of 1490 μg/ml. RCP clearly failed to scavenge free radicals and therefore showed poor inhibition percentages and the highest IC$_{50}$ value.

RCL was most efficient out of all, owing to its highest % inhibition at 1000 μg/ml, 143.54 %, which was comparable to that of Ascorbic acid 130.4% at the same concentration. The IC$_{50}$ value calculated for RCL was only 154.6 μg/ml, which was almost 1/3rd of that for Ascorbic acid (625 μg/ml)

The IC$_{50}$ values for RCF, RCS and RCR are 198.7, 204.79 and 297.34 (μg/ml) respectively.
4.1.3.3 Metal ion scavenging

Table 4.3 has showcased the metal chelating behavior of the various extracts of *R. communis*. IC$_{50}$ values of RCL, RCF, RCS, RCR and RCP were 77.5 μg/ml, 290 μg/ml, 188 μg/ml, 179.45 μg/ml and 589.7 μg/ml respectively; and that of the standard EDTA was 162.82

However, out of all, the highest percent inhibitions were observed in case of the RCL, 117.41±1.153 and 215.37±2.18 at 200 μg/ml and 1000 μg/ml respectively; and an IC$_{50}$ value less than half of that of EDTA.

RCP showed an exceptionally poor metal ion scavenging ability with the highest IC$_{50}$, 589.7 μg/ml; and lowest inhibition, 38.07±5.8 % at 200μg/ml concentration. However, its inhibition % at 1000 μg/ml was comparable to the standard’s inhibition % at 200 and 400μg/ml being 60.68 ± 0.02 and 69.9 ± 0.08 respectively. Thus, proving that RCP might have had some antioxidant property at higher concentrations.

From the results gathered, the antioxidant property of the various extracts of the plant is evidently proved. Metal ion scavenging percentages of RCL and RCS are significantly higher than that of the standard EDTA at all the five concentrations studied.

4.1.3.4 SOD activity

The *in vitro* SOD activity of various hydro-ethanolic extracts of *R. communis* was expressed as units per mg of protein in Table 4.4.

It could be noted that activity of RCL was 46.3± 1.6 at 200 μg/ml, 102.2±5.7 at 600 μg/ml and **182.6±8.17 at 1000 μg/ml**.

The activity was found to be 39.4±4.105, 116±6.196 and 172±8.994 at 200, 600 and 1000 μg/ml respectively in case of RCS. The same for RCF were observed as 41.2±1.564, 85±6.092 and 131.0±21.451 respectively. RCR exhibited 27.9±11.654, 71.9±41.788 and 118.2±92.656 at 200, 600 and 1000 μg/ml respectively.

RCP showed the lowest SOD activity of 23.4±0.88, 36± 2.1 and 52± 3.4 at 200, 600 and 1000 μg/ml respectively.
Results

From the observations it could be concluded that both RCL and RCS showed highest specific activities, being 182.6±8.17 μ/mg and 172.0±8.994 μ/mg respectively at concentration 1000 μg/ml.

4.1.3.5 CAT activity

Table 4.4 enlists the in vitro Catalase activities of all hydro-ethanolic extracts of *R. communis* as μM H₂O₂ per min per mg protein.

The specific activity values in μ/mg at 200 μg/ml and 1000 μg/ml of various extracts were noted to be 6.87 ± 2.30 and 16.87± 3.5 RCL; 5.42 ± 3.85 and 14.72± 2.3 for RCF; 5.97 ± 2.2 and 15.98± 7.3 for RCS; 4.86 ± 1.87 and 14.27± 3.23 for roots and 1.2±0.6 and 6.7± 3.46 for RCP respectively.

The results again revealed that the leaf and the stem extracts exhibited the highest potential for CAT with 16.87± 3.5 μ/mg and 15.98± 7.3 μ/mg respectively.

4.1.3.6 GPx activity

The GPx enzyme activity of each hydro-ethanolic extract of *R. communis* is depicted in Table 4.4 as units per mg protein.

The values for RCL were 73±5.9 μ/mg at 200μg/ml and 180±5.3 μ/mg at 1000 μg/ml concentration proved that it has the best activity as compared to all the extracts, followed by RCS with activity of 46±6.4 μ/mg and 162±3.9 μ/mg at 200 and 1000 μg/ml.

After these, the RCF and RCR showed activities of: 24±4 μ/mg, 123±1.4 μ/mg (RCF) and 34±5.4 μ/mg and 116±2.1 μ/mg (RCR) at 200 and 1000 μg/ml respectively.

The lowest specific activity values were recorded in case of RCP with 18.7± 2.22μ/mg at 200μg/ml and 116±2.1 μ/mg at 1000 μg/ml.

4.2 Evaluation and comparison of the antihyperglycemic and antidyslipidemic potential of various hydroethanolic extracts of different parts of *R. communis* in alloxan-induced diabetic male adult Swiss albino mice.

In this part of the study, various hydro-ethanolic extracts of *R. communis* have been used to treat alloxan-induced diabetic male Swiss albino mice. Their treatment had been
compared to that of standard drug (Glibenclamide) and with untreated normal and diabetic mice.

**Experiment:** Experimental animals had been divided into seven groups of seven mice each as per Table 3.2. Administration of a single intraperitoneal injection of alloxan at a dose of 150mg/ kg BW to all groups was done except NC. Thereafter, animals with fasting blood glucose level more than 140 mg/ dl after one week of alloxan administration confirmed their being diabetic and were selected for further study. 10 mg/ kg BW Glibenclamide and 300 mg/kg BW of various hydro-ethanolic extracts were administered orally once a day to the experimental groups for a period of 45 days. After completion of 45 days of treatment, all experimental groups were kept for overnight fasting. Blood was collected by retroorbital puncture after which the mice were sacrificed by cervical dislocation for the collection of selected organs (liver, kidneys and pancreas), which were used for further examination.

**4.2.1 Morphological effects**

Table 4.5 depicts the effect of alloxan, standard drug and various extracts on the body weight of the experimental animals during 45 days of treatment.

It was clearly visible that there was non-significant change in the mean body weight of mice of group NC from zero to 45th day. Whereas, all other groups observed a moderate stage of polyurea, polyphagia and polydypsia just after the induction of diabetes and displayed a sudden loss in their body weights. The percentage decrement in the body weights from before induction to after induction of alloxan were 17.17% in DC, 9.7% in GT, 7.9% in RCLT, 9.5% in RCFT, 7.1% in RCST and 11.41% in case of RCRT. Some animals also showed aggressive behavior and fur fall.

The 45 day treatment in GT, RCLT, RCFT, RCST seemed helpful in significantly ($P<0.05$) increasing the lowered levels body weights but however failed to restore their normal values. On the other hand, RCRT showed its adverse effect by further depreciating the mean body weight of the animals to only 22.0 ± 1.6 which was 16.28% decrease.
Results

All the treatments were found successful in rectifying other symptoms to a certain extent after the experimental duration. These symptoms persisted till the end in animals of group DC.

Thus the data showed that post alloxan administration there was a loss in the body weight. But glibenclimide and extracts RCL, RCF and RCS treatment showed positive effect on the weight loss in 45 days duration

4.2.2 Effect of extracts of *R. communis* on various biochemical parameters

This heading describes the consequences of 45 days treatment of standard drug glibenclamide and various extracts of plant on various biochemical parameters of alloxan induced, diabetic, adult swiss albino male mice.

4.2.2.1 Fasting Blood Glucose (mg/ dl)

A tell a tale of FBG levels of the experimental mice is given in table 4.6 FBG values under 80mg/dl were considered hypoglycemic; values in between 80-140 mg/ dl were considered under the normal range; whereas, glucose levels more than 140 mg/dl blood were taken to be diabetic.

According to the tabulated results, the blood glucose levels of all the groups were more or less the same before induction. However, the after induction values varied with different groups and time duration.

Unlike group NC where the FBG level of all the animals remained in the normal range from the zero to 45th day, all other groups exhibited a drastic increment in their blood glucose concentrations just after induction. Thus, at zero day, animals of all the groups except NC were highly diabetic with FBG values of 185.57±13.91 depicting a 44.86 % increase in DC, 206±35.37 ie. 51.5% increment in GT, 186±3.43 or 43.39 % escalation in case of RCLT, 180±19.4 a 38.8% increase in RCFT, 189±44.4 marking a 42.1% shoot in RCST and 42.79% appraisal with 174.8±38.91 in RCRT

Different treatments followed different recovery patterns on FBG levels till the end of experiment. RCRT showed an exceptional a hypoglycaemic effect by displaying rapid decrease in FBG levels, the lowest being 68 ± 17.98 (61.37%) on the 45th day. RCLT also
showcased a powerful antihyperglycemic activity by bringing down the outraged FBG levels to 89.92 ± 8.73 (51.6 %) which was comparable to the effect of GT 95.7 ± 18.1 (53.8 %) on the 45th day. RCFT and RCST displayed marked decrement by bringing down the FBG levels to 114.28± 10.87 (36.4%) and 125.6±12.6 (33.01) respectively.

4.2.2.2 Serum total protein (gm/dl)
Observations related to the effects of crude extracts and glibenclamide on serum total proteins after the whole 45 days study has been presented in table 4.8, which depicts that the amount of total proteins in group NC is 7.4±0.62 which was almost halved by the effect of alloxan in diabetic animals as its value for DC is 3.35±0.50. The standard drug appreciably elevated the lowered protein content to 8.87±0.6 ie.: By 62.2% which was significantly greater than that of NC.
All the other treatments either restored or improved the protein content values of 9.2±0.1 by 63.5 % for RCLT, 8.6±0.4 by 61% in group RCRT, 7.81± 0.2 by 57.1 % in the case of RCST and 7.1±0.07 by 52.8 % in RCFT.
Thus it can be said, RCLT was most effective than GT, whereas, the effect of RCRT on total proteins was comparable to GT.

4.2.2.3 Total lipid profile (mg/ dl)
The data enlisted in table 4.7 briefs the account of variations taking place in the lipid profile of experimental animals after the effect of diabetes induction by alloxan and further treatment by glibenclamide and the four hydro-ethanolic crude extracts.
A significantly (P<0.05) profound elevation of the total cholesterol (TC) was noticed after induction from 98.42± 13.9 to 230 ± 13.4, a 57.2% increase (DC) which was significantly (P<0.05) lowered by 35.2% in GT (149.71± 9.3); almost normalized significantly (P<0.05) by RCLT and RCRT, by bringing the values to 109.6 ± 9.4 (52.34%) and 84.2 ± 6.65 (63.3%) respectively. RCFT and RCST also lowered down the cholesterol levels to 151.3±3.6 (34.2%) and 132.57 ± 7.5 (42.3%) respectively; their effect was comparable to that of glibenclamide, while the effect of RCRT brought the cholesterol levels lower than the normal value.
Another lipid profile parameter triglyceride which was found to be considerably increased significantly \((P<0.05)\) from 113.86 ± 14.6 in NC to 171.71 ± 15.29 in DC; was significantly reduced to 71.14 ± 5.2, 86.8 ± 10.1, 66.9 ± 30.1, 105.43 ± 12.5 and 48.8 ± 41.6 in glibenclamide, RCLT, RCFT, RCST and RCRT respectively.

High density lipoprotein (HDL), one of the very important lipid profile parameters was reduced significantly \((P<0.05)\) in diabetic control group, however, their level is substantially improved in glibenclamide as well as various crude extract treated groups.

Low density lipoproteins (LDL) level significantly \((P<0.05)\) saw a marked increase of 79.67% in DC as compared to NC. The abnormally increased values were lowered significantly \((P<0.05)\) in glibenclamide by 53.3% but still did not normalize the levels. Whereas, RCLT was found successful in approximately restoring the normal LDL levels by 79.29%. RCRT led to an out of the way highlighted dip of 95.7%.

Likewise, very low density lipoproteins (VLDL) estimation showed a very high and significant increment after alloxan administration from 22.85 ± 2.96 in NC to 34.28 ± 2.9 in DC. But 45 days treatment with standard drug glibenclamide and also with crude extracts approximately normalized it towards normalcy such that the observed low level were 14.29 ± 1.11, 17.4 ± 2, 13.38 ± 6, 21 ± 2.5 and 9.8 ± 8.3 for GT, RCLT, RCFT, RCST and RCRT respectively.

From the data it could be inferred that treatment with all the four crude extracts and glibenclamide was efficient to certain extent in lowering the elevated levels of lipid profile parameters with the only exception of RCRT which exerted exceptionally adverse effects.

### 4.2.2.4 Hepatic glycogen content (mg/ gm tissue)

Observations outlined in table 4.9 clearly showed that the effect of diabetes induction by alloxan in mice significantly \((P<0.05)\) reduced the hepatic glycogen content from 45.89 ± 12.7 (NC) to 19.30 ± 8.24 (DC) which was a marked 57.9% decrement. However, the various treatments lead to commendable increase in the lowered glycogen levels. The most positive was the standard drug, glibenclamide treated whose value was 97.62 ± 20.8 (80.2% higher) ; followed by RCRT which showed a glycogen level of 84.68 ± 12.08
Results

(77.2% rise); the next being RCLT 81.25 ± 16.42; RCST 77.42 ± 9.56 and RCFT with 62.30 ± 3.27 which was respectively 76.2%, 75% and 69.02% more.

Thus all the treatments studied were capable of not only restoring but improving the total hepatic glycogen content.

4.2.2.5 Tissue total protein content (mg/ml tissue homogenate)

The content of tissue total protein in all the three organs is recorded in Table: 4.10 It is clearly evident that induction of DM with alloxan led to ablation of protein content by 65.94%, 55.57% and 52.17% in liver, kidney and pancreas respectively. However, the various treatments improved the decreased protein level at different efficiencies. For instance, GT showed 12.3%, 25.91% and 11.76% increment in liver, kidney and pancreas; all the four crude extracts showed almost similar percentages of increment of the tissue protein level, the most efficient being the leaf extract which exhibited 66.9% increase in liver, 60.96% increase in kidney and 51.47% increase in pancreas.

4.3 Comparative analysis of in vivo antioxidant potential of hydroethanolic extracts of various parts of R. communis and Glibenclamide on diabetes induced oxidative stress and the histopathological changes in various organs of the most efficient crude extract treated group.

This part discusses the aftermath of treatment of diabetic, male, Swiss albino mice by various hydro-ethanolic extracts of R. communis and glibenclamide, biochemically and histopathologically under the following headings:

4.3.1 In vivo antioxidant potential

4.3.1.1 Effect on lipid peroxidation in liver, kidney and pancreas experimental mice.

The trend of variations in the lipid peroxidation level (nm TBARS/mg Protein) in various organs post-experimental period is described in details in table 4.11. The values of group DC clearly indicated the significant (P<0.05) amount of hideous turnover of lipid peroxidation in all the organs viz., 406.2 ± 16.9 from 27.2± 4.4 (93.30%) in liver; 417.14
Results

± 92.6 from 58.18 ± 9.07 (86.05%) in kidney and 102.77± 14.8 from 7.35± 1.28 (92.84%) in the pancreas.

Glibenclamide treatment helped in bringing down the TBARS content till 134.51± 24.8 (68.87% decrease) in liver tissue homogenate, 21.6 ± 13.98 (78.87% decrease) in kidney and 107.8 ± 5.2 (76.68% lower) in case of pancreas.

The RCLT showed the most potent anti-lipid peroxidative ability by significantly (P<0.05) dropping down the elevated level to normalcy. The TBARS calculated in liver after RCLT treatment were 26.3 ± 2.7 (93.52% decrement), 54.67±0.71 (86.89.% decrease) in kidney and 9.4±0.4 (90.84% depreciation) in pancreas.

4.3.1.2 Effect on SOD activity in experimental mice after 45 days

Data presented in table 4.11 depicts the effect of alloxan administration and subsequent treatment of various extracts on the level of SOD in hepatic, renal and pancreatic tissues in Units/min/mg Protein. Data shows that alloxan annoyance resulted in significant (P<0.05) ablation of SOD activity till 26.02% in liver, 37.43% in kidney and 10.39% in pancreas.

Treatment with glibenclamide did not significantly (P<0.05) normalize rather further lowered the SOD activity by 5.75% till 132.6± 2.51 in liver and by 22.2% upto 113.8±9.13 in kidney. It however showed a different trend in pancreas by slightly motivating the activity by 5.27% to 208.4± 15.9.

RCFT enhanced the lowered SOD level in liver and kidneys significantly (P<0.05) to 178.4±15.5 and 129 ± 3.4, while reduced those in pancreas to 181.1±11.9.

Similar significant (P<0.05) trend of escalation in liver, kidneys by 20.4% and 12.8% respectively in case of RCST and 21.98% and 1.54% in case of RCRT while lowering in pancreas by 14.6% (RCST), 55.77% (RCRT) was seen.

RCLT however was significantly (P<0.05) the most successful in elevating the depleted SOD level by restoring them approximately in all the organs liver, kidney as well as in pancreas with 25.75% (189.5± 45.07), 19.7% (182.3 ± 6.02) and8.99% (216.9±9.72) increase respectively.

All the treatments failed to curb the fall in pancreatic SOD level except for RCLT.
4.3.1.3 Effect on CAT activity in experimental mice after 45 days

Values representing the effect of alloxan induced diabetes and subsequent co-administration of standard drug/herbal preparation on CAT is compiled in table 4.1 in μmoles H$_2$O$_2$ decomposed/min/mg Protein. It is quite evident that marked decline in the activity of CAT is seen after alloxan administration, as the value of CAT activity which was 208.2±9.43, 268±2.64 and 214.3± 8.9 in liver, kidney and pancreas of group NC respectively dipped significantly (P<0.05) to about 136.3± 6.6(34.5%), 146.4± 5.7 (45.37%) and153.3±2.85 (28.46%) respectively in case of DC.

All the hydro-ethanolic extract treatments except RCRT displayed not only a restorative but significant (P<0.05) beneficial effect on the CAT level in liver: 372.1±2.7 (63.3%) by RCLT, 223.09±7.5 (62.24%) by RCFT, 293.7±5.4 (47.8%) by RCST; kidney: 421.6±16.6 (80.02%) in RCLT, 342.8±10.52 (75.42%) in RCFT and 378.6±1.49 (77.75%) in RCST as well as in pancreas: 315.2±8.5 (51.36%) by RCLT, 288±2.6 (46.77%) by RCFT and 245±11 (34.42%) by RCST).

RCRT also showed enhancement of CAT level as compared to DC but failed to restore them as in NC, being 178.8± 4.7, 23.7% more than DC; 219.13±6.7 61.56% higher than DC and 205±6.1, 21.25% elevated than DC in liver, kidney and pancreas respectively.

Similarly the standard drug also exhibited uplifting the values but only slightly. The values of CAT activities recorded for GT in liver, kidney and pancreas were 150.5±7.8, 196.5±3.9 and 194.2±9.5 respectively.

4.3.1.4 Effect on GPx activity in experimental mice after 45 days

Status of GPx activity in μg GSH consumed/min/mg Protein in untreated, alloxan treated and alloxan co-administered various herbal extracts or glibenclamide have been presented in table 4.11 Data informs a significant (P<0.05) declination of 43.58% in liver (from 217.5±12.7 to 122.7 ± 10.3), of 48.28% in pancreas (from 172.1 ± 22.3 to 89.01 ± 9.7) and of 49.80% in kidney (from 167.8 ±8.6 to 84.23 ± 12.17) after alloxan administration.

After 45 days of Glibenclamide administration the GPx contents significantly (P<0.05) elevated to 148.4±6.5 in liver(17.31%), decreased to 96.2±5.7 (22.54%) in kidney and
elevated to 105.16±20.2 (15.35%) in pancreas. However, in kidney this drug brought further depreciation in the activity of this enzyme.

Further, 45 days treatment with crude extracts of leaves, stems, roots and flowers proved significantly (P>0.05) successful in improving the activity of GPx in liver, kidney and pancreas. Leaves extract treatment to group RCLT inflated the GPx content by 46.58% in liver, 36.66% in kidney and 50.88% in pancreas.

Increment by RCFT and RCST was found to be 34.3% and 32% respectively in pancreas, 24.3% and 30.44% respectively in kidney and 25.54% and 19.54 respectively in liver tissues.

In roots treated group, the elevation observed was 38.25% (144.16±9.9) in kidney and only 0.96% (123.9±10.3) in liver whereas in pancreas it resulted in further decrease till 73.5±20.2.

4.3.1.5 Effect on GSH in experimental mice after 45 days

Observations elucidating an exact picture of hepatic, renal and pancreatic GSH content (mg/gm tissue) in different experimental groups have been described in table 4.11 and it can be clearly noticed that significantly (P<0.05) lowered amount of hepatic (12.3±1.8), renal (8.7±2.3) and pancreatic (3.6±0.4) GSH are recorded in group DC when compared to NC (24.6±4, 24±3.6 and 7.8±0.4 respectively)

However when treatment with glibenclamide and the four herbal extracts were taken into account, it was found that GT helped in elevating the content to almost twice to that of NC ie: 19.2±1.65 significantly (P<0.05) in case of liver, to 17.8±3.4 in kidney and 7.5±0.78 in pancreas.

The improved significant (P<0.05) level by RCLT, RCFT and RCST were 27.1±8.17, 30.7±7.23 and 24.65±1.6 in liver; 27.0±3.94, 18.6±1.28 and 21.8±3.66 in kidney and 9.20±4.3, 9.6±2.7 and 8.6±4.1 in case of pancreas.

Treatment with RCRT was unsuccessful in elevating the GSH content with values 4.83±0.78, 8.96±11.27 and 4.2±13.6 in liver, kidney and pancreas respectively.
4.3.1.6 Effect on Serum albumin in experimental mice after 45 days

Table 4.8 depicts the level of albumin in mg/ gm tissue in various control and treated experimental groups. The data collected revealed that alloxan administration resulted in 61.9 % (4.2 ± 0.11 to 1.6 ± 0.1) decline in albumin content of DC. Glibenclamide treatment significantly (P<0.05) elevates the level to 2.87 ± 0.25, a 44.25% rise. Crude extract resulted in significant (P<0.05) mounting the level post alloxan injection near to normalcy with 4.7± 0.2 (65.9%), 5± 0.05 (68%), 2.7± 0.1 (40.7%) and 4.5±0.7 (64.44%) in RCLT, RCFT, RCRT and RCST respectively.

4.3.1.7 Effect on Serum globulin in experimental mice after 45 days

Data in table 4.8 demonstrates the consequences (serum globulin in mg/ gm tissue) of various treatments i.e., of glibenclamide, crude extracts of *R. communis* and various controls. Post alloxan administration, the globulin level decreased down to 47.5 % (3.2 ± 0.2 to 1.68± 0.2). Glibenclamide escalated the level slightly by 20% to 2 ± 0.3. The crude extracts proved beneficial in rising the globulin level after alloxan injection. The level of serum globulin increased to 3.8 ± 0.07 (55.78%) by leaves, 3.9 ± 0.03 (56.9%) by flowers, 2.9 ± 0.2 (44.82%) in roots and 3 ±0.2 (46.66%) in case of stem extract treatment. All the results were significant at P<0.05.

4.3.2 Effect on histopathological changes in various organs after 45 days experiment.

This part of study emphasises on the 45 days treatment of standard drug Glibenclamide and the most efficient crude extract i.e.; hydro-ethanolic leaf extract of plant *R. communis* on histopathology of liver, pancreas and kidney of alloxan induced swiss albino mice. Results obtained have been presented here.

4.3.2.1 Liver

Alloxan administration to the mice of group DC leads to the many abrasions in the liver such as dilation in the sinusoids, infiltration in portal triad, granular cytoplasm
Results

degeneration and neutrophillic infiltration (fig 4.23) as compared to the normal control group (fig 4.20). However RCLT (fig 4.29) resulted in normalizing almost all the parameters. Standard drug (fig 4.26) is capable in successfully recovering the anomaly caused by alloxan treatment. Crude herbal extracts however recover almost all deformaties in tissues, but some tissues show glycogen infiltration and minor necrosis.

4.3.2.2 Kidney
Histopathological observations in the present study indicated that alloxan induction to DC resulted in the shrinking of the glomerulus along with arteriolar thickening (fig 4.24) as compared to that of NC (fig.4.21). But after the 45 days treatment with the extracts, all the deformities were normalized in glibenclamide treatment (fig 4.27), extract treatment (fig 4.30). Thus, concluding that the leaf extract has the ability to restore the abnormalities caused by alloxan injection.

4.3.2.3 Pancreas
In this study, pancreatic tissue of group NC shows normal islets of langerhans (fig 4.22). But after the alloxan administration, damaged islets of langerhans were observed (fig 4.25). However extract treatment resulted in normalising the deformities caused by alloxan (fig 4.31). An interesting striking feature observed in the glibenclamide treated group (fig 4.28) was presence of granulomatous reactions in the lymph nodes adjacent to pancreas. Granulomatous reactions are the immune based disease that can cause cell mediated immunity (CMI).

From these results, it can be concluded that *R. communis* not only removed almost all deformities both biochemically as well as histopathologically

4.4 Isolation, identification, purification of active principle compound from the Leaf extract and Evaluation of its effects on diabetes induced oxidative stress on experimental animals.

Among the hydro-ethanolic extracts of the four parts of *R. communis* evaluated for their anti-hyperglycemic and anti-oxidative potential, the leaf extract was found to be the most successful. The preliminary phyto-chemical analysis revealed that the leaf extract exhibited the presence of alkaloids and flavanoids in high amounts, which was assumed
to be responsible for its beneficial medicinal properties. However, particular compounds that help in alleviating the hyperglycemia and related oxidative stress is still ambiguous. This part of the study therefore, stresses upon:

1. The isolation of compounds from the hydro-ethanolic leaf extract of *R. communis*
2. *In vivo* evaluation of their antihyperglycemic and anti-oxidative efficacies.
3. Characterization and purification of the most active antihyperglycemic and anti-oxidative compound isolated.

4.4.1 The isolation of compounds from the hydro-ethanolic leaf extract of *R. communis*

4.4.1.1 TLC (Thin Layer Chromatography) analysis

The leaf extract was analyzed on silica gel G plates with various mobile phases of different polarities. The solvent system Ethyl acetate: Acetone: Formic acid (9: 6: 1) revealed the presence of maximum number of compounds; 6 compounds (corresponding to the 6 distinct spots in (fig 4.35). These were named as C1, C2, C3, C4, C5 and C6 having R\textsubscript{F} value of 0.46, 0.70, 0.76, 0.83, 0.93 and 0.96 respectively (Table 4.1). The chromatograms when developed in Iodene chamber yielded six different mustard colored spots. On treatment with Dragendorff’s reagent, FeCl\textsubscript{3} and Erlich’s reagent the spots C4 and C1 gave reddish brown to black grey coloration.

4.4.1.2 Column Chromatography analysis and confirmatory TLC

After streamlining the solvent system isolating the maximum number of compounds in TLC, almost same mobile phase with slight modification Ethyl acetate: Acetone: Formic acid (9: 6: 0.5) was used to run a column packed with the leaf extract bound Silica gel G beads, for proper isolation and purification of the compounds. The fractions were run on TLC with different polarity mobile phases to confirm the singularity of the isolated product. Out of the 120 fractions collected all were further compared for components using TLC with Ethyl acetate: Acetone: Formic acid (9: 6: 1) mobile phase. Some fractions showed absence of any spots, mixing of two or more spots, formation of faint spots etc on confirmatory TLC analysis. However, fig 4.36 and table 4.13 showed that
fraction number 112-115 resulted into distinct, single, prominent spots with $R_F$ value similar to C4 isolated from TLC, while fractions 45-47 lead to development of bold, single spots with $R_F$ value same as C1 on confirmatory TLC. All the spots obtained from both the set of fractions showed positive response to Iodene, Dragendorf’s reagent, FeCl$_3$ and Erlich’s treatment. Plus the phytochemical analysis for C1 and C4 were found positive for the presence of alkaloids.

4.4.2 In vivo evaluation of antihyperglycemic and anti-oxidative efficacies of C1 and C4

The fractions collected were dried and dissolved in Tween 20 for orally treating Alloxan induced diabetic male mice for a period of 45 days. The morphological, biochemical and histopathological changes noted in and after the experimental period as compared to the normal, diabetic untreated, diabetic glibenclamide treated and diabetic Leaf extract treated groups are as follows:

4.4.2.1 Morphological changes

Table 4.5 depicts the effect of alloxan, standard drug and various extracts on the body weight of the experimental animals during 45 days of treatment. The treatment with Alloxan led to a significant decrease in body weights of experimental mice. However, the treatments with various isolated compounds from leaves of *R. communis* motivated the body weights positively. C4 caused an increment in the body weight by 13.1%, C1 led to a 7.05% increase while the elevation by RCL crude extract was only 2.42%.

Some animals also showed aggressive behavior and fur fall which were rectified by both the treatments.

4.4.2.2 Biochemical changes

4.4.2.2.a Effect of 45 days experiment on FBG level

Table 4.6 clearly gives a picture of the effect of 45 days experiment on FBG level. Induction of DM with Alloxan resulted in massive, significant rise in fasting blood glucose level of mice under the study. This elevation was lowered by subsequent
treatments: Glibenclamide led to a 35.8% decrease. The crude leaf extract RCL potentially reduced the heightened FBG by 51.6%. Whereas, the isolated compounds C1 and C4 significantly lowered the value by 55.47% and 10.5% respectively.

4.4.2.2.b Effect of 45 days experiment on Total Serum Protein content
The effect of 45 days experiment on Total Serum Protein content is well depicted in Table 4.8
The alloxan induction led to an 18.9% decrement in serum total protein value in DC. This was restored by 47.8% with Glibenclamide treatment, 18.33% with C1 treatment and 31.6% on treating the diabetic mice with C4.

4.4.2.2.c Effect of 45 days experiment on lipid profile
A detailed description of lipid profiles effected by the 45 day treatment with the isolated compounds is given in Table 4.7.
A significantly (P<0.05) profound elevation of the total cholesterol (TC) was noticed after Alloxan induction from 98.42±13.9 to 230±13.4, a 57.2% increase (DC) which was significantly (P<0.05) lowered by 35.2% in GT (149.71±9.3); almost normalized significantly (P<0.05) by 42.30% and 18.6% in C1T and C4T respectively.
High density lipoprotein (HDL), one of the very important lipid profile parameters was reduced significantly (P<0.05) from 40.86±13.3 in NC to 24.4±5.47 in diabetic control group, however, their level is substantially improved in glibenclamide (55.71±1.5) as well as in C1T and C4T(45.82±2.5 and 32±4.3 respectively).
Another lipid profile parameter triglyceride which was found to be considerably increased (significant at P<0.05) from 113.86±14.6 in NC to 171.71±15.29 in DC; was significantly reduced to 71.14±12.5, 94.6±5.6, and 164.16±39 in glibenclamide, C1T and C4T respectively.

4.4.2.2.d Effect of 45 days experiment on Hepatic glycogen content
From table 4.9 it can be said that the normal glycogen level of 45.89±12.7 mg/g tissue was severely reduced by 57.94% after alloxan induced diabetes in DC. The herbal
preparations showed not only restorative but motivated the glycogen levels higher than the normal by 80.2% in case of GT, 73.9% in C1T and 68.13% in C4T.

4.4.2.2.e Tissue total protein content (mg/ml tissue homogenate)

The content of tissue total protein in all the three organs is recorded in Table: 4.10 It is clearly evident that induction of DM with alloxan led to ablation of protein content by 65.94%, 55.57% and 52.17% in liver, kidney and pancreas respectively. However, the various treatments improved the decreased protein level at different efficiencies. For instance, GT showed 12.3%, 25.91% and 11.76% increment in liver, kidney and pancreas. The most efficient improvement in case of isolated compounds was observed in C1 treated group which increased the protein content by 64.57% in liver, 51.66% in kidney and 54.16% in pancreas. Whereas, the improvement by C4 was 63.73%, 58.31% and 43.10%, in liver, kidney and pancreas respectively.

4.4.2.2.f Effect of 45 days experiment on antioxidant status

Table 4.11 elaborates the effect on the antioxidant status of LPo, SOD, CAT, GPx and GSH in various organs of experimental mice.

4.4.2.2.f.i Effect on Lipid peroxidation in Liver, Kidney and Pancreas of experimental mice.

The values of group DC clearly indicated the significant (P<0.05) amount of hideous turnover of lipid peroxidation in all the organs viz., 406.2 ± 16.9 from 27.2± 4.4 (93.30%) in liver; 417.14 ± 92.6 from 58.18 ± 9.07 (86.05%) in kidney and 102.77 ± 14.8 from 7.35± 1.28 (92.84%) in the pancreas.

Glibenclamide treatment helped in bringing down the TBARS content till 321.4± 24.8 (26.38% decrease) in liver tissue homogenate, 290.52 ± 3.98 (30.35% decrease) in kidney and 85.5 ± 5.2 ( 16.74% lower) in case of pancreas.

The C1 treatment showed enormous 87.44%, significant (P<0.05) depreciation in the diabetic TBARS calculated in liver, by 94.8% in kidney and by 93.8% in pancreas.
Treatment with C4 was also effective in bringing down the TBARS but not as efficiently as C1.

**4.4.2.2.f.ii Effect on SOD in Liver, Kidney and Pancreas of experimental mice.**

The administration of alloxan lead to a 26.02% dip in the SOD activity in the liver which was only ridiculed further by 6.10% in GT, motivated by 27.36% by C1 treatment and only slightly enhanced in group C4T by 11.9%

The kidneys underwent similar detoration of 26.24% in diabetic non treated group, while GT further reduced the SOD status by 22.2%. C1T on the other hand motivated by only 4.4%. The C4T however almost restored the normal SOD activity in the kidney.

In case of pancreas the dip after alloxan treatment in DC was 54.01% which was risen slightly by 5.2% in GT, 26.34% in C1T and 20.15% in C4T.

**4.4.2.2.f.iii Effect on CAT in Liver, Kidney and Pancreas of experimental mice.**

The activity of CAT enzyme also observed reduction in diabetic mice as compared to the normal control mice, in all the three organs, precisely 34.53% in liver, 68.6% in kidney and 28.46% in pancreas.

The treatment with GT only slightly helped the activity by 9.43% in liver, majorly by 57.1% in kidney, and again 21.06% in pancreas.

The positive effect of both the isolated compounds were 57.05% and 30.77% by C1T and C4T respectively in liver; by 78.71% (C1T) and 71.76% (C4T) in kidney and by 34.83% and 24.14% respectively in C1T and C4T, in pancreas.

**4.4.2.2.f.iv Effect on GPx in Liver, Kidney and Pancreas of experimental mice.**

Diabetic GPx activity was seen to reduce by 43.58%, 25.98% and 42.28% in liver kidney and pancreas respectively.

GT caused 17.31% increase in liver, 22.5% decrease in kidney, 15.26% increment in case of pancreas
The treatment with C1 resulted in higher GPx activity as compared to that by C4 being, 23.4% increase in liver, 33.15% rise in kidney, and a slight 16.8% higher in pancreas (as compared to the group DC)

4.4.2.2.f.v Effect on GSH in Liver, Kidney and Pancreas of experimental mice.

The hepatic tissue saw notable lowering in GSH level from $39.2 \pm 5.0$ in NC to $12.3 \pm 1.8$ in DC (ie: 68.62% decrease). Treatment with C1 motivated GSH to $25.6 \pm 8.4$ (51.9% higher) and that with C4 enhanced it to $23.6 \pm 7.24$ (46.61% more).

Alloxan induction caused a 68.2% depreciation of normal GSH in renal tissue which was best restored by C1T with 63.52% rise.

Pancreatic GSH was reduced by 34.45% in DC, restored by 25.71% in C1 treated and decreased further by 2.56% in C4 treated mice.

4.4.2.2.f.iv Effect on globulin

The normal $3.2 \pm 0.2$ mg/dl globulin content was reduced to $1.2 \pm 0.2$ in diabetic mice, which was elevated to $2.4 \pm 0.8$ in C1T and $3.1 \pm 0.1$ in C4T groups. (Table 4.8)

4.4.2.2.f.vii Effect on Albumin

The $4.2 \pm 0.11$ mg/dl albumin content in NC was lowered to $1.6 \pm 0.1$ by the effect of alloxan in DC, this was restored to $3.8 \pm 0.3$ and $4.7 \pm 1.1$ mg/dl in C1T and C4T respectively. (Table 4.8)

4.4.2.3 Histopathological changes

The most efficient isolated compound, C1 was successful in restoring all the alloxan-induced deformities at histological level, and this was visible in all the three organs (Liver, Kidney, Pancreas) studied.

4.4.3 Characterization and purification of the most active antihyperglycemic and anti-oxidative compound isolated C1.

The *in vivo* evaluation of the isolated compounds revealed that C1 was more effective than C4 in restoring the blood glucose level, antioxidant status and histopathological
changes in Alloxan induced diabetic mice. Therefore, the characterization and purification of the isolated compound C1 was carried out as follows:

4.4.3.1 Characterization of C1 by spectrophotometric analysis

IR spectra (fig 4.37, 4.38) confirmed the presence of NH group. The presence of C-C bonds was also prominently evident. Other functional groups such as C=O, aromatic groups were also indicated.

NMR spectra (4.39) depicted the presence of an acetic acid (or a derivative) linked to the C3 carbon atom of an indole showing ring as well as acidic protons.

The GC-MS (4.40) revealed a molecular ion peak at 175.1 m/z; predicting it to be Indole acetic acid.

4.4.3.2 HPLC profile of crude extract, IAA standard and C1 for purity determination

The qualitative HPLC profile of crude extract of R. communis leaves (fig 4.41), isolated compound C1 (fig 4.42) and the Indole acetic acid standard (fig 4.43) were detected at a wavelength of 282 nm due to sharpness of the peaks and proper baseline and recorded its retention time (R,t min), percent area and heights were recorded. The HPLC chromatogram of the crude extract has shown 12 peaks. However, only 3 peaks were prominent with significant percent area and height (> 0.5%). One of the most prominent peaks with 556.9105 area and amount 4.28004 ng/µl is observed at the retention time 5.915 min, which is somewhat similar to that observed in case of C1 and the standard. It is probably a Protoalkaloid present in the extract.

However, in the HPLC chromatogram of C1, only one prominent peak was visible with area 5040.00146 Whose R,t was found to be 5.957 (min). In the chromatogram apart from this prominent peak, a few conspicuous peaks were also detected, which may be attributed to the presence of certain impurities in small concentration along with the isolated compound. (fig 4.42)
Likewise, in the HPLC chromatogram of standard IAA (fig 4.43), I prominent peak was witnessed with an area of 1.301, at 10 ng/µl, at the retention time of 6.035 (min), which to a certain extent corresponds with that of C1.

Thus, the results of HPLC confirmed the alkaloid nature of the isolated compound C1.

4.5 Genetic expression analysis of various antioxidant enzyme genes in liver and kidney of mice

In order to study the relative expression of SOD, CAT, GPx and GSR mRNA in liver and kidneys of normal, diabetic untreated, diabetic and Glibenclamide treated and diabetic and Leaf extract treated mice, RNA was isolated and was checked for its purity and concentration (Table 4.14). These RNA were further used to prepare quality cDNA which produced single bands at 320 bp with GAPDH (Figure: 4.44). The primers designed for amplification of SOD, CAT, GPx and GSR genes were prepared using Primer3 tool (Table 4.15) and were optimized using gradient PCR technique. Figures 4.45-4.48 show gel images for Primer optimization. After this, RT PCR experiment was performed and $2^{-\Delta\Delta C_T}$ method was applied for its analysis.

The internal housekeeping gene was used as GAPDH, while the calibrator selected for the study was normal, untreated Kidney and normal, untreated Liver.

Using the $2^{-\Delta\Delta C_T}$ method, the data are presented as the fold change in gene expression normalized to an endogenous reference gene and relative to the untreated control, for which $\Delta\Delta C_T$ equals zero and $2^0$ equals one, so that the fold change in gene expression relative to the untreated control equals one, by definition. For the treated samples, evaluation of $2^{-\Delta\Delta C_T}$ indicates the fold change in gene expression relative to the untreated control.

4.5.1 Expression of SOD gene:

The normalized abundance of SOD mRNA to GAPDH mRNA showed (Fig. 4.49) that there was significantly (P<0.01) higher expression of SOD in case of normal tissues, both liver and kidney. However, with the introduction of diabetes, the expression significantly (P<0.01) dipped by 92.3% in kidney and by 78.8% in liver. The Glibenclamide treatment,
lead to a significant 38.48% rise in kidney while no effect was noted in mRNA of liver. The Leaf extract therapy motivated the expression in both liver and kidney by 84.83% and 85.64%

NCK > RCLK > GTK > DCK

NCL > RCLL > GTL > DCL

4.5.2 Expression of CAT gene

The significant (P<0.01) alterations in expression of CAT described in Figure 4.52 are different as compared to the other genes as the fold change of mRNA in normal liver is 24.18% more than that in the calibrator, normal kidney. The expression of CAT in untreated diabetic kidney and liver was respectively reduced by 98.33% and 128.97%. This decrease was very minutely elevated by 6.7% and 6.69% in kidney and liver respectively by Glibenclamide treatment. The leaf extract motivated an increment of 95.58% in kidney and 97.78% in liver.

NCK > RCLK > GTK > DCK

NCL > RCLL > GTL > DCL

4.5.3 Expression of GPx gene

Figure 4.50 overviews the significant (P<0.01) comparative fold change in the expression of the GPx gene under study. The data revealed that, fold change of GPx mRNA in normal untreated liver was lesser as compared to the calibrator. The fold change values were slashed by 93.75% and 87.5% in kidney and liver tissues respectively owing to the diabetes induction by Alloxan. Rather than helping the expression values, Glibenclamide only added to the depreciating fold change by 59.95% in kidney and 50.06% in liver. On the other hand, the leaf treatment elevated the reduced fold change value in kidney by 90.51% and 89.25% in liver. So much so that the fold change of mRNA of leaf treated liver tissues was 14.1% more than that in the normal untreated liver.

NCK > RCLK > DCK > GTK
the other hand, the leaf treatment elevated the reduced fold change value in kidney by 90.51% and 89.25% in liver. So much so that the fold change of mRNA of leaf treated liver tissues was 14.1% more than that in the normal untreated liver.

\[
\text{NCK > RCLK > DCK > GTK} \\
\text{RCLL > NCL > DCL > GTL}
\]

4.5.4 Expression of GSR gene

The variations in the fold change of GSR mRNA are noted in Figure 4.51. The expression of GSR in liver tissues of untreated mice is same as that in calibrator. The Alloxan introduction significantly (P<0.01) ridiculed these values by 99.55% in kidney and 99.4% in liver tissue. Treatment with Glibenclamide did no good to elevate or restore the expression of GSR, it only slightly raised it in kidney by 6.66%, insignificantly (P<0.01) and further lowered it in liver by 59.32%. Unlike Glibenclamide, the leaf extract exerted some relief by significantly (P<0.01) increasing the fold change of GSR mRNA by 99.36% and 98.9% in kidney and liver tissue respectively.

\[
\text{NCK > RCLK > GTK > DCK} \\
\text{NCL > RCLL > DCL > GTL}
\]
Table 4.1:

Phyto-chemical constituents in various extracts
<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>RCL</th>
<th>RCF</th>
<th>RCP</th>
<th>RCS</th>
<th>RCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Fatty Acids</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Steroids</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Tannins</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Terpeoids</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 4.1:
Table 4.2: *In vitro* anti-diabetic activities of various extracts.

Standard used: Acarbose

The values are Mean ± SD (n=3); the values are significantly different at p<0.05
<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc (µg/ml)</th>
<th>% inhibition of diabetic enzymes by various extracts of <em>R. communis</em></th>
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</thead>
<tbody>
<tr>
<td>α-amylase activity</td>
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<tr>
<td>200</td>
<td>27.02 ± 2.309</td>
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</tr>
<tr>
<td>400</td>
<td>37.27 ± 22.38</td>
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<tr>
<td>600</td>
<td>47.7 ± 19.42</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>61.51 ± 3.687</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>74.99 ± 3.270</td>
<td></td>
</tr>
<tr>
<td>IC₅₀(µg/ml)</td>
<td>602 320 805 401 668 325.50</td>
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<tr>
<td>α-glucosidase activity</td>
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<tr>
<td>200</td>
<td>29.87 ± 3.88</td>
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<tr>
<td>400</td>
<td>40.93 ± 4.44</td>
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<tr>
<td>600</td>
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</tr>
<tr>
<td>800</td>
<td>57.93 ± 6.810</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>63.78 ± 7.282</td>
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<tr>
<td>IC₅₀(µg/ml)</td>
<td>617 348 988 598 624 230.71</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2
Table 4.3: *In vitro* free radical scavenging activities

The values are Mean ± SD (n=3); the values are significantly different at p<0.05.

Standards used: Ascorbic acid was used as standard for ABTS and DPPH radical scavenging activities; EDTA was used as standard for metal ion chelation activity.
Table 4.3

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<tr>
<th>Free Radical scavenging Activities</th>
<th>Concentration (μg/ml)</th>
<th>RCF</th>
<th>RCL</th>
<th>RCP</th>
<th>RCR</th>
<th>RCS</th>
<th>Standard</th>
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</thead>
<tbody>
<tr>
<td>ABTS radical scavenging</td>
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<td>65.96±4.58</td>
<td>28.7±1.9</td>
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<td>42.34±2.8</td>
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<td></td>
<td>400</td>
<td>64.72±3.9</td>
<td>83.79±4.2</td>
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<td>80.25±2.704</td>
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<td>97.596±3.5</td>
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<td></td>
<td>600</td>
<td>94.3±12.3</td>
<td>125.6±6.6</td>
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<td>121.3±5.4</td>
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<td>160.3±4.8</td>
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<td>162.15±2.02</td>
<td>134.98±9.76</td>
<td>154.74±4.64</td>
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<tr>
<td></td>
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<td>182.14±7.632</td>
<td>224.98±6.2</td>
<td>56.6±1.87</td>
<td>206.59±2.7</td>
<td>174.13±9.49</td>
<td>180.51±6.37</td>
</tr>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>292.8</td>
<td>185.2</td>
<td>802</td>
<td>197.4</td>
<td>230.7</td>
<td>146.3</td>
</tr>
<tr>
<td>DPPH radical scavenging</td>
<td>200</td>
<td>53.02±2.9</td>
<td>68.1±1.12</td>
<td>4.7±8.8</td>
<td>43.2±2.2</td>
<td>48.7±7.3</td>
<td>6.87±3.88</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>65.27±2.8</td>
<td>84.84±3.471</td>
<td>8.9±3.4</td>
<td>59.09±6.1</td>
<td>68.58±5.1</td>
<td>21.33±2.34</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>88.7±1.2</td>
<td>111.88±7.9</td>
<td>14.4±2.6</td>
<td>76.98±8.6</td>
<td>82.116±7.2</td>
<td>46.78±7.8</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>101.1±8.7</td>
<td>127.81±18.05</td>
<td>24.09±2.7</td>
<td>91.28±1.7</td>
<td>98.93±8.7</td>
<td>87.3±8.78</td>
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<td>1000</td>
<td>129.3±6.7</td>
<td>143.54±7.7</td>
<td>32.8±7.4</td>
<td>113.8±5</td>
<td>134.39±1.25</td>
<td>130.4±10.18</td>
</tr>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>198.7</td>
<td>154.6</td>
<td>1490</td>
<td>297.34</td>
<td>204.79</td>
<td>625</td>
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<tr>
<td>Metal ion chelation</td>
<td>200</td>
<td>35.58±8.49</td>
<td>117.41±1.153</td>
<td>38.07±5.8</td>
<td>40.32±1.30</td>
<td>71.82±9.58</td>
<td>60.68±0.02</td>
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<tr>
<td></td>
<td>400</td>
<td>69.67±2.37</td>
<td>143.73±6.60</td>
<td>45.6±6.7</td>
<td>98.38±0.312</td>
<td>86.21±8.463</td>
<td>69.9±0.08</td>
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<td>600</td>
<td>102.6±8.31</td>
<td>162.26±7.8</td>
<td>52.4±7.4</td>
<td>124.62±1.39</td>
<td>112.25±11.2</td>
<td>83.78±0.07</td>
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<tr>
<td></td>
<td>800</td>
<td>129.30±8.17</td>
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<td>147.63±88.1</td>
<td>136.66±1.05</td>
<td>95.9±0.07</td>
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<td></td>
<td>1000</td>
<td>137.09±2.68</td>
<td>215.37±2.18</td>
<td>67.6±5.1</td>
<td>162.68±2.2</td>
<td>179.13±8.11</td>
<td>104.87±0.02</td>
</tr>
<tr>
<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>290</td>
<td><strong>77.5</strong></td>
<td>589.7</td>
<td>179.45</td>
<td>188.34</td>
<td>162.82</td>
</tr>
</tbody>
</table>
Table 4.4: Specific activities of enzymatic antioxidants *in vitro*

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

Units of measurement were: Units m^{-1}g^{-1} or units per mg protein
<table>
<thead>
<tr>
<th>Specific Activity of Enymatic Antioxidants</th>
<th>Conc (μg/ml)</th>
<th>Sample</th>
<th>Sample</th>
<th>Sample</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RCF</td>
<td>RCL</td>
<td>RCP</td>
<td>RCR</td>
</tr>
<tr>
<td>Superoxide Dismutase SOD</td>
<td>200</td>
<td>41.2±1.564</td>
<td>46.3±1.6</td>
<td>23.4±0.88</td>
<td>27.9±11.654</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>66.0±2.062</td>
<td>73.8±2.74</td>
<td>29±1.3</td>
<td>53.3±10.495</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>85±6.092</td>
<td>102.2±5.7</td>
<td>36±2.1</td>
<td>71.9±41.788</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>116.5±4.745</td>
<td>146.8±2.531</td>
<td>43±2.6</td>
<td>97.5±7.440</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>131.0±21.451</td>
<td>182.6±8.17</td>
<td>52±3.4</td>
<td>118.2±92.656</td>
</tr>
<tr>
<td>Catalase CAT</td>
<td>200</td>
<td>5.42±3.85</td>
<td>6.87±2.30</td>
<td>6.87±2.30</td>
<td>4.86±1.87</td>
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<tr>
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<td>400</td>
<td>8.36±3.5</td>
<td>9.74±2.5</td>
<td>9.74±2.5</td>
<td>7.66±8.87</td>
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<td>600</td>
<td>10.66±7.35</td>
<td>12.5±5.0</td>
<td>12.5±5.0</td>
<td>9.66±8.87</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>12.46±3.5</td>
<td>14.6±2.3</td>
<td>14.6±2.3</td>
<td>12.6±0.7</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>14.72±2.3</td>
<td>16.87±3.5</td>
<td>16.87±3.5</td>
<td>14.27±3.23</td>
</tr>
<tr>
<td>Glutathione peroxidase GPx</td>
<td>200</td>
<td>2.4±4</td>
<td>7.3±5.9</td>
<td>1.87±2.22</td>
<td>3.4±5.4</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>5.6±0.09</td>
<td>10.5±3.0</td>
<td>2.37±5.8</td>
<td>5.4±3.5</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>7.5±3.8</td>
<td>12.3±2.2</td>
<td>2.56±0.8</td>
<td>6.9±2.02</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>10.6±8.05</td>
<td>15.8±7.5</td>
<td>2.89±4.66</td>
<td>9.4±1.10</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>12.3±1.4</td>
<td>18.0±5.3</td>
<td>3.46±7.8</td>
<td>11.6±2.1</td>
</tr>
</tbody>
</table>

Table 4.4
Table 4.5: Effect of 45 days experiment on Body Weight of mice.

Values are mean ± SEM of 7 observations.

*Before induction (Basal values); Students’ ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to basal values; b: significant ($P<0.05$) difference compared to values obtained after alloxan injection; b*: insignificant difference ($P>0.05$) compared to values obtained after alloxan injection.

Table 4.6: Effect of 45 days experiment on Fasting Blood Glucose (FBG) level in mice.

Values are mean ± SEM of 7 observations.

*Before induction (Basal values); Students’ ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to basal values; b: significant ($P<0.05$) difference compared to values obtained after alloxan injection; b*: insignificant difference ($P>0.05$) compared to values obtained after alloxan injection.
<table>
<thead>
<tr>
<th>TIME DURATION</th>
<th>BODY WEIGHT OF IN GRAMS (GMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>NC</td>
</tr>
<tr>
<td>BEFORE INDUCING</td>
<td>27.4 ± 2.3</td>
</tr>
<tr>
<td>AFTER INDUCING 0 DAY</td>
<td>27 ± 2.4</td>
</tr>
<tr>
<td>22nd DAY</td>
<td>27.2 ± 2.1</td>
</tr>
<tr>
<td>45th DAY</td>
<td>26.9 ± 0.5 b*</td>
</tr>
</tbody>
</table>

Table 4.5

<table>
<thead>
<tr>
<th>TIME DURATION</th>
<th>FASTING BLOOD GLUCOSE IN MG/DL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>NC</td>
</tr>
<tr>
<td>BEFORE INDUCING</td>
<td>106.14 ± 13.18</td>
</tr>
<tr>
<td>AFTER INDUCING 0 DAY</td>
<td>106 ± 25.4a*</td>
</tr>
<tr>
<td>22nd DAY</td>
<td>102.4b* ± 29.4</td>
</tr>
<tr>
<td>45th DAY</td>
<td>103.71 ± 9.8b*</td>
</tr>
</tbody>
</table>

Table 4.6
Table 4.7: Effect of 45 days experiment on Lipid Profile parameters

Values are mean ± SEM of 7 observations.

Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b*: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c*: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.
<table>
<thead>
<tr>
<th>LIPI D PARAMETERS</th>
<th>Lipid Profile in mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>NC</td>
</tr>
<tr>
<td>HIGH DENSITY LIPOPROTEIN</td>
<td>40.86 ± 13.3</td>
</tr>
<tr>
<td>LOW DENSITY LIPOPROTEIN</td>
<td>34.71 ± 21.02</td>
</tr>
<tr>
<td>VERY LOW DENSITY LIPOPROTEIN</td>
<td>22.85 ± 2.96</td>
</tr>
<tr>
<td>TRIGLYCERIDE</td>
<td>113.86 ± 14.6</td>
</tr>
<tr>
<td>TOTAL CHOLESTROL</td>
<td>98.42 ± 13.9</td>
</tr>
</tbody>
</table>

Table 4.7
**Table 4.8: Effect of 45 days experiment on Serum Proteins**

Values are mean ± SEM of 7 observations.

Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b*: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c*: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.

**Table 4.9: Effect on Hepatic Glycogen Content after 45 days experiment.**

Values are mean ± SEM of 7 observations.

Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b*: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c*: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.
### Table 4.8

<table>
<thead>
<tr>
<th>GROUPS</th>
<th><strong>HEPATIC GLYCOGEN CONTENT</strong> (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROL</strong></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>45.89 ± 12.7</td>
</tr>
<tr>
<td>DC</td>
<td>19.30 ± 8.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>TREATED</strong></td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>97.62 ± 20.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCF</td>
<td>84.68 ± 12.08&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCL</td>
<td>81.25 ± 16.42&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCR</td>
<td>62.30 ± 3.27&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCS</td>
<td>77.42 ± 9.56&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>C1</td>
<td>74.00 ± 9.56&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>C4</td>
<td>60.56 ± 7.04&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table 4.9

<table>
<thead>
<tr>
<th>GROUPS</th>
<th><strong>SERUM PROTEIN CONTENT</strong> (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL PROTEIN</strong></td>
<td><strong>ALBUMIN</strong></td>
</tr>
<tr>
<td>6.5-8.2</td>
<td>3.5-5</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>7.4 ± 0.62</td>
</tr>
<tr>
<td>DC</td>
<td>6 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>TREATED</strong></td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>8.87 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCF</td>
<td>7.1 ± 0.07&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCL</td>
<td>8.1 ± 0.1&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCR</td>
<td>9.6 ± 0.4&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCS</td>
<td>7.81 ± 0.2&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIT</td>
<td>7.1 ± 0.07&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>C4T</td>
<td>7.9 ± 0.6&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> Significantly different from control group; <sup>b</sup> Significantly different from treated group; <sup>bc</sup> Significantly different from control and treated groups.
Table 4.10: Effect of 45 days experiment on Tissue Total Proteins

Values are mean ± SEM of 7 observations.

Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b*: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c*: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.
<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Tissue Total Proteins (mg/ml tissue homogenate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>CONTROL</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>14.77 ± 2.4</td>
</tr>
<tr>
<td>DC</td>
<td>5.03 ± 1.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TREATED</td>
<td></td>
</tr>
<tr>
<td>GT</td>
<td>5.74 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCF</td>
<td>12.43 ± 3.7&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCL</td>
<td>15.2 ± 2.65&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCR</td>
<td>12.9 ± 4.6&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>RCS</td>
<td>14.67 ± 3.1&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>C1</td>
<td>14.2 ± 2.3&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>C4</td>
<td>13.87 ± 0.87&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 4.10
Table 4.1: Antioxidant status of mice Liver, Kidney and Pancreas after 45 days experiment

Values are mean ± SEM of 7 observations.

Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a*: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b*: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c*: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.

Units of measurement: LPo is expressed in nm TBARS/mg Protein; SOD activity in Units/ min/ mg Protein; CAT activity in μmoles/ min/ mg Protein; GPx activity in μg/ min/ mg Protein; GSH level in mg/ gm Tissue.
<table>
<thead>
<tr>
<th>Antioxidant Parameter</th>
<th>Organ</th>
<th>Group</th>
<th>LPo</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC</td>
<td>27.2 ± 4.4</td>
<td>190.2±15.4</td>
<td>208.2 ±9.43</td>
<td>217.5 ±12.7</td>
<td>39.2 ± 5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DC</td>
<td>406.2 ±16.9^a</td>
<td>140.7±5.1^a</td>
<td>136.3±6.6^a</td>
<td>122.7±10.3^a</td>
<td>12.3±1.8^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT</td>
<td>321.4 ±24.8^ab</td>
<td>132.6±2.5^ab^x</td>
<td>150.5±7.8^ab</td>
<td>148.4±6.5^ab</td>
<td>19.2 ±1.65^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCF</td>
<td>58.47±9.7^abc</td>
<td>178.4±15.5^abc</td>
<td>214.4±9.8^abc</td>
<td>196.5±23^abc</td>
<td>30.7±7.23^abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCL</td>
<td>26.3 ± 2.7^abc</td>
<td>189.5±45.07^abc</td>
<td>372.1±2.7^abc</td>
<td>229.73±7.5^abc</td>
<td>27.1 ± 8.17^abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCR</td>
<td>111.51±0.3^abc</td>
<td>162.68± 7.5^abc</td>
<td>178.8 ± 4.7^abc</td>
<td>123.9 ±10.3^abc</td>
<td>4.83 ±0.78^abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCS</td>
<td>58 ± 2.2^abc</td>
<td>175.9 ±7.8^abc</td>
<td>293.7 ±5.4^abc</td>
<td>152.5 ± 0.69^abc^x</td>
<td>24.65 ±1.6^abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1T</td>
<td>51.01 ± 1.4^abc</td>
<td>193.7 ± 1.5a^bc</td>
<td>317.4± 9.8^abc</td>
<td>160.2 ±7.5^abc</td>
<td>25.6 ±8.04^abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C4T</td>
<td>31.7±5.2^abc</td>
<td>159.71 ± 4^abc</td>
<td>196.9 ± 8.65^abc</td>
<td>155.82 ± 8.3^abc</td>
<td>23.6 ±7.24^abc</td>
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<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td>NC</td>
<td>58.18 ± 9.07</td>
<td>198.5 ±26.07</td>
<td>268 ± 2.64</td>
<td>167.8 ±8.6</td>
<td>27.36±8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DC</td>
<td>417.14 ± 92.6^a</td>
<td>124.2 ± 5.7^a</td>
<td>146.4 ±5.7^a</td>
<td>84.23±12.17^a</td>
<td>8.7±2.3^a</td>
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<tr>
<td></td>
<td></td>
<td>GT</td>
<td>290.52 ±3.98^b</td>
<td>113.8±9.13^ab^x</td>
<td>196.5±3.9^a^b</td>
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<td>17.8 ±3.4^ab</td>
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<tr>
<td></td>
<td></td>
<td>RCF</td>
<td>47.28 ± 5.9^abc</td>
<td>129 ± 3.4^abc</td>
<td>285 ± 4^abc</td>
<td>178.6 ± 13.6^abc^x</td>
<td>18.6 ±1.28^abc</td>
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<tr>
<td></td>
<td></td>
<td>RCL</td>
<td>54.67 ±0.71^abc</td>
<td>182.3±6.02^abc^c^x</td>
<td>421.6±16.6^abc</td>
<td>184.46±15.5^abc</td>
<td>27.0 ±3.94^abc</td>
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<td></td>
<td>RCR</td>
<td>90.68±1.26^abc</td>
<td>148.7 ± 6.5^abc^c</td>
<td>219.13 ± 6.7^abc</td>
<td>144.16±9.90^abc^x</td>
<td>21.8±3.66^abc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCS</td>
<td>71.15±3.91^abc</td>
<td>167.9±3.5^abc</td>
<td>378.6±1.49^abc</td>
<td>178.56±14.68^abc^x</td>
<td>8.96±11.27^abc</td>
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<tr>
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<td></td>
<td>C1T</td>
<td>21.6 ± 2.53^abc</td>
<td>153.2 ± 5.8^abc</td>
<td>395.8 ±3.25^abc</td>
<td>185.81±20.35^abc</td>
<td>23.85±5.53^abc</td>
</tr>
<tr>
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<td>C4T</td>
<td>62.3±7.9^abc</td>
<td>194.09 ±0.19^abc</td>
<td>298.7±2.8^abc</td>
<td>76.8 ± 4.05^abc</td>
<td>19.8 ±6.8^abc</td>
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<tr>
<td><strong>Pancreas</strong></td>
<td></td>
<td>NC</td>
<td>7.35 ±1.28</td>
<td>220.3 ±14.5</td>
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<td>11.9 ±4.4</td>
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<td></td>
<td>DC</td>
<td>102.77 ±14.8^a^b</td>
<td>197.4 ±27.9^a</td>
<td>153.3 ± 2.85^a</td>
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<td>GT</td>
<td>85.5 ± 5.2^abc</td>
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<td>7.5 ±0.78^abc</td>
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<td>RCF</td>
<td>60.8±0.09^abc</td>
<td>181.1±11.9^abc</td>
<td>224±17.6^abc</td>
<td>128.4±7.8^abc</td>
<td>9.6±2.7^abc</td>
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<td>RCL</td>
<td>9.4 ± 0.4^abc</td>
<td>216.9±9.7^abc^c</td>
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<td>9.20 ±4.3^abc^x</td>
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<td>87.3 ±13.9^abc</td>
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<td>73.5 ±20.2^abc</td>
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<td>RCS</td>
<td>52.9 ±0.36^abc</td>
<td>168.4±9.5^abc</td>
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<td>6.36 ±12.8^abc</td>
<td>174 ± 5.3^abc</td>
<td>298±12.9^abc^x</td>
<td>126.4 ± 13.9^abc</td>
<td>10.5±7.8^abc</td>
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<td></td>
<td>C4T</td>
<td>8.2±2.6^abc</td>
<td>166.4 ±4.2^abc^c</td>
<td>256±6.6^abc</td>
<td>105.8±10.0^abc</td>
<td>7.6 ±1.4^abc</td>
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Table 4.11
Table 4.12: TLC analysis

Table 4.13: TLC confirmatory analysis
<table>
<thead>
<tr>
<th>SNo</th>
<th>Compound isolated</th>
<th>R_F value</th>
<th>Color of spot</th>
<th>No treatment</th>
<th>FeCl₃ treatment</th>
<th>Dragendorff’s treatment</th>
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<tbody>
<tr>
<td>1</td>
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<td>0.46</td>
<td>Mustard green</td>
<td>Blue-black</td>
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<tr>
<td>2</td>
<td>C2</td>
<td>0.70</td>
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<td>No color</td>
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<td>0.76</td>
<td>Light yellow</td>
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<td>No color</td>
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<tr>
<td>4</td>
<td>C4</td>
<td>0.83</td>
<td>Light green</td>
<td>Grey green</td>
<td>Grey</td>
<td></td>
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<tr>
<td>5</td>
<td>C5</td>
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<tr>
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Table 4.12

<table>
<thead>
<tr>
<th>SNo</th>
<th>Fractions Collected</th>
<th>R_F Value</th>
<th>Corresponding Compound Isolated</th>
<th>Color of spot with Dragendorff's treatment</th>
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<tr>
<td>1</td>
<td>45-47</td>
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<td>C4</td>
<td>Brown</td>
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<td>112-115</td>
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<td>C1</td>
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Table 4.13
Table 4.14: Concentration and Purity of isolated RNA

Table 4.15: Primers designed for genetic expression analysis
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<thead>
<tr>
<th>SNo</th>
<th>Sample</th>
<th>Concentration(ng/μl)</th>
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<th>260/230</th>
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<td>NK</td>
<td>2775.2</td>
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<tr>
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<td>DCK</td>
<td>3743</td>
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<td>1.93</td>
</tr>
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<tr>
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<td>3476.6</td>
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<tr>
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<td>RCLL</td>
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<td>1.94</td>
<td>1.81</td>
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<td>8</td>
<td>RCLK</td>
<td>3786.4</td>
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<td>2.02</td>
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Table 4.14

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<th>GC%</th>
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<td>5’GATGCCTGCTTCACCACCTTCT3’</td>
<td>62</td>
<td>54</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>GSR F</td>
<td>3’-CACCGAGGAACCTGGAGAATG-5’</td>
<td>60.6</td>
<td>55</td>
<td>20</td>
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<tr>
<td>4</td>
<td>GSR R</td>
<td>5’-ATCCGTCTGAATGCCCACT-3’</td>
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<td>52.38</td>
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<td>9</td>
<td>CAT F</td>
<td>3’-CCCCCAACTATTACCCAAC-5’</td>
<td>60</td>
<td>55</td>
<td>20</td>
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<tr>
<td>10</td>
<td>CAT R</td>
<td>5’-TTTCTCTCCTCCTCGTTCA-3’</td>
<td>59</td>
<td>50</td>
<td>20</td>
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Table 4.15
Figure: 4.1

Percentage inhibition of α-amylase by various extracts of *R. communis*

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

Figure: 4.2

Percentage inhibition of α-glucosidase by various extracts of *R. communis*

The values are Mean ± SD (n=3); the values are significantly different at p<0.05
Figure 4.1

Figure 4.2
Figure: 4.3

Percentage inhibition of ABTS radicals by various extracts of *R. communis*

The standard used was Ascorbic acid

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

Figure: 4.4

Percentage inhibition of DPPH radicals by various extracts of *R. communis*

The standard used was Ascorbic acid

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

Figure: 4.5

Percentage inhibition of metal ions by various extracts of *R. communis*

The standard used was EDTA

The values are Mean ± SD (n=3); the values are significantly different at p<0.05
Figure 4.6

*In vitro* specific activity of SOD

Units of measurement were: units per mg protein

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

---

Figure 4.7

*In vitro* specific activity of CAT

Units of measurement were: units per mg protein

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

---

Figure 4.8

*In vitro* specific activity of GPx

Units of measurement were: units per mg protein

The values are Mean ± SD (n=3); the values are significantly different at p<0.05
Figure 4.9

Effect of 45 days experiment on Body Weight of mice.

Values are mean ± SEM of 7 observations.

*BI: Before induction (Basal values); Students’ ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to basal values; b: significant ($P<0.05$) difference compared to values obtained after alloxan injection; b: insignificant difference ($P>0.05$) compared to values obtained after alloxan injection.

Figure 4.10

Effect of 45 days experiment on Fasting Blood Glucose (FBG) level in mice.

Values are mean ± SEM of 7 observations.

*BI: Before induction (Basal values); Students’ ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to basal values; b: significant ($P<0.05$) difference compared to values obtained after alloxan injection; b: insignificant difference ($P>0.05$) compared to values obtained after alloxan injection.
Figure 4.9

Figure 4.10
Figure 4.11

Effect of 45 days experiment on Serum Proteins

Values are mean ± SEM of 7 observations.
Units of measurement: mg/dl
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.

Figure 4.12

Effect of 45 days experiment on Lipid Profile parameters

Values are mean ± SEM of 7 observations.
Units of measurement: mg/dl
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.
Figure 4.11

Figure 4.12
Figure 4.13

Effect on Hepatic Glycogen Content after 45 days experiment.

Values are mean ± SEM of 7 observations.
Units of measurement: mg/g tissue
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, $\alpha$: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, $\beta$: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, $\gamma$: insignificant difference ($P>0.05$) compared to Glibenclamide Treated

Figure 4.14

Effect on Tissue Total Protein content in various organs after 45 days experiment.

Values are mean ± SEM of 7 observations.
Units of measurement: mg/ml tissue homogenate
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, $\alpha$: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, $\beta$: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, $\gamma$: insignificant difference ($P>0.05$) compared to Glibenclamide Treated
Figure 4.13

Figure 4.14
Figure 4.15

Effect on Lipid Peroxidation in various organs after 45 days experiment.

Values are mean ± SEM of 7 observations.
Units of measurement: nm TBARS/mg Protein
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated

Figure 4.16

Effect on SOD activity in various organs after 45 days experiment.

Values are mean ± SEM of 7 observations.
Units of measurement: Units/ min/ mg Protein
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated
Figure 4.15

Figure 4.16
**Figure 4.17**

**Effect on CAT activity in various organs after 45 days experiment.**

Values are mean ± SEM of 7 observations.
Units of measurement: µmoles/min/mg Protein
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.

**Figure 4.18**

**Effect on GPx activity in various organs after 45 days experiment.**

Values are mean ± SEM of 7 observations.
Units of measurement: µg/min/mg Protein
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated.
Figure 4.19

Effect on GSH Content in various organs after 45 days experiment.

Values are mean ± SEM of 7 observations.
Units of measurement: mg/ gm Tissue
Student’s ‘t’-test is significant at $P<0.05$. a: significant ($P<0.05$) difference, a: insignificant difference ($P>0.05$) compared to Normal Control; b: significant ($P<0.05$) difference, b: insignificant difference ($P>0.05$) compared to Diabetic Control; c: significant ($P<0.05$) difference, c: insignificant difference ($P>0.05$) compared to Glibenclamide Treated
Figure 4.19
Figure 4.20
Photomicrograph of transverse section of Normal Control Liver (400X)

Figure 4.21
Photomicrograph of transverse section of Normal Control Kidney (400X)

Figure 4.22
Photomicrograph of transverse section of Normal Control Pancreas (400X)
Figure 4.23

Photomicrograph of transverse section of Diabetic Control Liver (400X)

Figure 4.24

Photomicrograph of transverse section of Diabetic Control Kidney (400X)

Figure 4.25

Photomicrograph of transverse section of Diabetic Control Pancreas (400X)
Figure 4.26

Photomicrograph of transverse section of Glibenclamide treated, diabetic Liver (400X)

Figure 4.27

Photomicrograph of transverse section of Glibenclamide treated, diabetic Kidney (400X)

Figure 4.28

Photomicrograph of transverse section of Glibenclamide treated, diabetic Pancreas (400X)
Figure 4.26

Figure 4.27

Figure 4.28
Figure 4.29

Photomicrograph of transverse section of RCL treated, diabetic Liver (400X)

Figure 4.30

Photomicrograph of transverse section of RCL treated, diabetic Kidney (400X)

Figure 4.31

Photomicrograph of transverse section of RCL treated, diabetic Pancreas (400X)
Figure 4.32

Photomicrograph of transverse section of C1 treated, diabetic Liver (400X)

Figure 4.33

Photomicrograph of transverse section of C1 treated, diabetic Kidney (400X)

Figure 4.34

Photomicrograph of transverse section of C1 treated, diabetic Pancreas (400X)
Figure 4.32

Figure 4.33

Figure 4.34
Figure 4.35

TLC analysis of compounds C1 to C6

Figure 4.36

Confirmatory TLC analysis of compounds C1 and C4
Figure 4.35

Figure 4.36
FTIR (Fourier Transform Infrared Radiation) analysis of isolated compound C1

Figure 4.37
Characterization of C1 by Infra Red Spectra (from wavenumber 4000-2000)

Figure 4.38
Characterization of C1 by Infra Red Spectra (from wavenumber 2000-500)
Figure 4.37

Figure 4.38
Figure 4.39

Characterization of C1 by NMR spectral analysis

Figure 4.40

Characterization of C1 by Gas Chromatography- Mass Spectroscopy (GC-MS)
Figure 4.39

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<td>175</td>
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Figure 4.40
Figure 4.41
HPLC chromatogram for crude extract

Figure 4.42
HPLC chromatogram for isolated compound C1
Figure 4.41

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Totals : 4.28004e-1

Figure 4.42

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<th>Amount</th>
<th>Grp</th>
<th>Name</th>
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<td>[min]</td>
<td>[mAU*s]</td>
<td>[ng/ul]</td>
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<td>7.68532e-4</td>
<td>3.87340</td>
<td>IAA</td>
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</tr>
</tbody>
</table>

Totals : 3.87340

Summed Peaks Report
Figure 4.43

HPLC chromatogram for standard Indole acetic acid
Figure 4.43
Figure 4.44

cDNA Synthesis

Figure 4.45:

Primer optimization for SOD
Figure 4.46:
Primer optimization for GPx

Figure 4.47:
Primer optimization for GSR

Figure 4.48:
Primer optimization for CAT
Figure 4.46:

Figure 4.47:

Figure 4.48:
Figure 4.49
Changes in the expression of SOD gene in Liver and Kidney of experimental mice
The values are Mean ± SD (n=3); the values are significantly different at p<0.05

Figure 4.50
Changes in the expression of CAT gene in Liver and Kidney of experimental mice
The values are Mean ± SD (n=3); the values are significantly different at p<0.05
Figure 4.49

Figure 4.50
Figure 4.51

Changes in the expression of GPx gene in Liver and Kidney of experimental mice

The values are Mean ± SD (n=3); the values are significantly different at p<0.05

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Figure 4.52

Changes in the expression of GSR gene in Liver and Kidney of experimental mice

The values are Mean ± SD (n=3); the values are significantly different at p<0.05
Figure 4.51

Fold change of GPx gene in Liver Sample

NCL  DCL  GTL  RCLL
1   0.125  0.0625  1.164

Fold change of GPx gene in Kidney Sample

NCK  DCK  GTK  RCLK
1   0.06   0.02   0.65

Figure 4.52

Fold change of GSR gene in Liver Sample

NCL  DCL  GTL  RCLL
0.0059  0.0024  0.543

Fold change of GSR gene in Kidney Sample

NCK  DCK  GTK  RCLK
0.0044  0.0048  0.7071