Chapter 5: DISCUSSION
5.1 Evaluation of phytochemical constituents and in vitro anti-diabetic and antioxidative potential of hydroethanolic extracts of *R. communis*.

5.1.1. Phytochemical screening:

Plants have always been valuable for medicinal purposes owing to the presence of some bioactive constituents including alkaloids, fatty acids, saponins, resins, phenols, steroids, terpenoids, glycosides, tannins, flavonoids etc, which provide various physiological action. Various phyto-constituents have been proven to exhibit different medicinal properties, for example: anti-diabetic, anti-cancerous, anti-inflammatory, anti-oxidative, antibacterial, anti-fungal, antiviral and many others (Neamsuvan *et al*., 2012; Edoga *et al*., 2013) Innumerable plants have been studied till date for their phyto-chemical nature, viz., *Carica papaya*, *Adenia cissampeloides* and *Cymbopogon citrates* (Njoku *et al*., 2004), *Carica papaya Linn*, *Magnifera indica Linn*. *Psidium guajava Linn*. *Vernonia amygdalina*(Ayoola *et al*., 2008), *Vernonia amygdalina*, *Sida acuta*, *Ocimum gratissimum* and *Telfaria occidentalis* (Adeniyi *et al*., 2010), *Bryophyllum pinnatum*, *Ipomea aquatica*, *Oldenlandia corymbosa*, *Ricinus communis*, *Terminalia bellerica*, *Tinospora cordifolia*, and *Xanthium strumarium* (Yadav and Agarwala, 2011), *Portulaca oleracea* (Okafor and Ezejindu, 2014), *Calotropis procera* (Tiwari *et al*., 2015)

The results from Table 1.1 depicted the presence of phytoconstituents viz., tannins, flavanoids, terpenoids, glycosides, phenols, saponins, alkaloids, steroids, fatty acids and resins in low (+), moderate (++) and high/ ample (+++) amounts.

Alkaloids are known to be one of the major phytoconstituents present in traditional plants for anti-fungal, anti-microbial, anti-diabetic, anti-cancerous, and anti-inflammatory activities (Okwu and Okwu, 2004). Alkaloids have also been reported for their cytotoxicity (Nobori *et al*., 1994), antispasmodic, and analgesic activity (Harbone, 1973; Nyarko and Addy, 1990). In this study RCL showed highest presence of Alkaloids thus it could be expected to cure diabetes. They were moderately present in RCP and RCR; and were present in low amounts in RCF and RCS.
Except all the extracts of the plant fatty acids were found present only in RCP. They were previously reported for their anti-fungal and anti-bacterial activities (Agoramoorthy et al., 2007).

**Resins** are considered as a good traditional medicinal source reported for the treatment of inflammation, microbial diseases, arthritis, for wound healing, against tumor and hyper-lipidemia (Shen et al., 2012). However, all the extracts of *R. communis* showed absence of Resins in them except the RCP where their presence was seen but in low amounts.

Flavonoids are known to possess antioxidant properties by demonstrating a variety of different pathways, such as free radical scavenging, metal (such as iron and copper) ion chelation and restriction of enzymes responsible for over-production of free radical (Benavente-Garcia, 1997). Usually higher phenol and flavanoid contents, lead to better scavenging activity, because of the availability of OH group in the polyphenols (Hatano, 1989; Vinson et al., 1995 and Ebrahimzadeh et al., 2009). Flavonoids along with phenols were also reported to inhibit the initiation, promotion and progression of tumors (Han et al., 2007). Both were potent water soluble antioxidants (Kim et al., 1994). These phenolic compounds also possess some biological properties to treat apoptosis, sepsis, carcinogens, ageing, inflammation, atherosclerosis and improvement of endothelial function and for cardiovascular protection (Okwu and Okwu, 2004). The anti-oxidative or free radical scavenging activity for scheming degenerative diseases were previously reported (Vani et al., 1997; Han et al., 2007).

The results established that RCL showed the presence of flavanoids in high amounts. They were present in moderate amounts in RCF, RCP and RCR. While in RCS the presence of Flavanoids was low. Phenols were found highest in RCS, moderately present in RCF and RCR, in low amounts in RCL and were missing in the pods. The hydro-ethanolic extracts of the plant were tested positive for saponins, moderately in RCL and RCS and found low in all other extracts. Presence of saponins in traditional plants was known to possess
ability to reduce cholesterol levels and could manage CVD in humans (Aletor, 1993). Saponins were also reported for their use as emulsifying agents and having anti-fungal (Osuagwu et al., 2007), anti-inflammatory activity (Just et al., 1998), coagulating and precipitating property in red blood cells. The extracts also showed moderate presence of steroids, except for RCF where they were lowly present and totally absent in RCP. Steroids are very important compounds and have been reported for antibacterial (Raquel, 2007) activity and their association with such compounds as sex hormones (Okwu, 2001).

Tannins suggested the ability to play a major role for the treatment of sore throat, wound healing, anti-diarrhoea and anti-haemorrhagic agents (Okwu and Okwu, 2004; Asquith and Butler, 1986). Tannins were also reported for the antimicrobial degradation of dietary proteins of semen (Aletor, 1993). They were moderately present in all the extracts under study, except for RCP.

Terpenoids were present in low amounts in RCL, RCF and RCR; moderately present in RCP and present in high amounts in RCS. These have carboxylic acid group due to which their presence was responsible for the activity of organic extracts (Antherden, 1969; Harborne, 1973). Glycosides were observed in every hydro-ethanolic extract of R. communis. Presence of glycosides make the plants responsible for lowering the blood pressure as previously they have been known to lower blood pressure (Nyarko and Addy, 1990)

Screening of various (ethyl acetate, ethanol and aqueous) extracts of the leaves of R. communis for the presence of phytochemicals by Obumselu et al., 2011 reported positive results for alkaloids (1.54%), saponins (0.46%), phenols, flavonoids (8.78%) and tannins (1.08 mg/ml).

The phytochemical analysis of ethanol extracts of Trigonella foenum graecum (seeds), Ricinus communis (leaves) and Delonix regia (flowers) was taken up by Khursheed et al., 2012. They concluded that presence of carbohydrates, alkaloids, phenols, proteins and tannins was common to all three plant extracts tested in different concentrations.
Phytochemical screening of methanolic extracts of *R. communis* had also been screened by Vandita *et al.*, 2013 showed the availability of saponins on one hand and the absence of tannins and flavonoids on the other.

Preliminary phytochemical screening studies were carried out by Sharma *et al.*, 2014 on various hydro-ethanolic extracts of *Pithecellobium dulce* and *Ricinus communis* for the presence of carbohydrates, tannins, proteins, glycosides, triterpenoids, alkaloids, steroids, flavonoids, saponins and phenols. The study revealed the extracts of *P. dulce* bark and *R. communis* leaves as the most phytochemically rich extracts. Chemical tests were also carried out by Okafor and Ezejindu in 2014 on the aqueous extract of aerial parts of *Portulaca oleracea* for the determination of their inclusive phyto-chemicals. The availability of Alkaloids, saponins, tannins, flavonoids, cardiac glycosides, terpenoids, steroids, phobatannins, proteins and starch was evaluated qualitatively whereas, the quantitatively determined phyto-constituents were flavonoids, tannins, alkaloids and saponins. It was assessed that its highest constituents were saponins, 32% and alkaloids, 26%. This study authenticated the use of *P. oleracea* for production of therapeutic drugs and to enhance its usage further for research-study.

Recently, in 2015 Shetty *et al.*, conducted a phytochemical study on stem of *Calotropis procera* and confirmed the availability of reducing sugars, tannins, proteins, glycosides, triterpenoids, alkaloids, steroids, flavonoids, saponins and phenols.

Thus, from the present study it could be deduced that among all the extracts RCL showed highest amount of alkaloids, flavanoids. RCS showed highest amount of phenols and terpenoids. RCR and RCS showed highest amount of glycosides. While only RCP showed a totally different pattern of absence of all other metabolites except the presence of fatty acids, that too in high amounts.
5.1.2. Anti-diabetic activity

The main physiological role of enzymes α-glucosidase and α-amylase is known to be the breakdown of starch ingested to maltose which is further broken into glucose for absorption and assimilation (Marshall, 1975; Soler-Rivas et al., 2000). These enzymes help by catalyzing the hydrolysis reaction of α-1, 4 glucosidic linkages of polysaccharides taken in diet. Therefore, their activity was enhanced in diabetics. Thus, food stuffs which inhibit such enzymes may prove to be of value as novel therapeutic, anti-diabetic agents (Jaffe and Lette, 1968). The hydro-ethanolic extracts of *R. communis* had shown a considerable inhibition of these enzymes, which was comparable to that of the standard Acarbose. The inhibition activities also followed an increasing trend with increasing concentrations.

5.1.2.1 α-glucosidase inhibiting ability of various extracts

α-glucosidase is an intestinal, carbohydrate digesting enzyme leading to the breakdown of starch and disaccharide into glucose. It usually acts upon the 1, 4-alpha bonds of sugars. The formation of glucose as a result of digestion of polysaccharides gives rise to hyperglycemic condition in the body causing diabetes (Nair et al., 2013). This enzyme also aids in the glucose absorption in the intestinal gut.

It could be deduced from the results gathered that the α-glucosidase inhibition activities of RCL and RCR were somewhat alike the standard, Acarbose. The IC$_{50}$ values of RCL and RCR were lowest, revealing them having higher anti-diabetic potential with respect to other extracts, whereas, RCP had the least anti-diabetic ability amongst all the five extracts studied. A decreasing order of α-glucosidase inhibition ability could be formed on the basis of observations:

Acarbose > RCL > RCR > RCF > RCS > RCP

5.1.2.2 α-amylase inhibiting ability of various extracts

α-amylase is an enzyme involved in digestion of large carbohydrates (e.g. glycogen, starch etc) linked with the alpha bonds. As a result high amount of glucose and maltose are formed. This leads to increased level of post prandial hyperglycemia, thereby, causing
serious diseased condition called as DM. Alpha-amylase basically acts on the alpha bond present in large polysaccharides. Hyperglycemic condition can be avoided with the help of α-amylase inhibitors (Nair et al., 2013).

From the results, it was drawn that the α-amylase inhibition activity of RCL was higher than that of Acarbose and that of RCR was comparable to that of the standard. Also, lowest IC₅₀ values of RCL and RCR revealed them as anti-diabetic with greater potential as compared to other extracts. While, RCP emerged out with lowest anti-diabetic potential amongst all extracts. Thus following trend of α-amylase inhibition could be concluded:

RCL> Acarbose> RCR> RCF> RCS> RCP

An investigation by Reddy et al., 2010 evaluated the in vitro antioxidative and antidiabetic effect of Asystasia gangetica leaves. The methanolic extract showed α-glucosidase (IC₅₀ - 325μg/ml) and α-amylase (IC₅₀ -3.75μg/ml) inhibitory activity which were reported to be concentration dependant. These results clearly indicated that the extract had significant antioxidative and α-glucosidase and α-amylase inhibitory potential.

Narkhede et al., 2011 screened the methanol extract of Caesalpinia digyna roots with the aim of assessing its antidiabetic activity, in vitro and reported the exhibition of increased inhibitory activity of α-glucosidase enzymes (IC₅₀ 402.23±10.14 μg/ml) and α-amylase (IC₅₀ of 686.94 ± 3.98 μg/ml), in a dose-dependent manner.

Methanolic extract of Psidium guajava (guava) leaves was investigated by Manikandan et al., in 2013, for its anti-diabetic ability. The study explained the vital role of digestive enzymes in digestion of carbohydrates. The plant showed significant inhibition activity of these enzymes and was thus recommended as a potent anti-diabetic operator.

Recently, in the year 2014, Verma et al., reported the in vitro bioefficacy of Hydro-ethanolic extracts of Ricinus communis bark and root for antioxidative and antihyperglycemic potential. All concentrations of both the extracts showed significantly (P < 0.05) higher activities than control. RC root exhibited IC₅₀ values of 401μg/ml for α-amylase and 598μg/ml for α-glucosidase which were lower as compared to RC bark. Their data presented, rationalized the hydro-ethanolic extracts of R. communis root and bark as potential remedies to treat hyperglycemia
5.1.3 Antioxidant ability assay:

The data presented, rationalized the bioefficacy of hydro-ethanolic extracts of *R. communis* in scavenging the free radicals and the potent enzymatic antioxidants.

- **Free radical scavenging potential**

Plant could be said to contain different antioxidants, it was however, difficult to measure each antioxidant separately therefore, several methods were employed to estimate the antioxidant ability. ABTS, DPPH and Metal Ion Scavenging methods were selected to assess the scavenging potential of all parts of *R. communis*.

5.1.3.1 ABTS scavenging

ABTS$^{•+}$ are generated from the pH - independent reaction of ABTS-e- and ABTS$^{•+}$ with ethanol or hydrogen donating groups to form 2, 2$^{’}$-azinobis (3-ethyl-benzothiazoline 6-sulfonate (ABTS) which is colourless. A decrease of the ABTS$^{•+}$ concentration was reported to be linearly dependent on the antioxidant concentration by Chanda and Dave, 2009.

From the results it was evident that the antioxidative activity of the extracts was very less than the activity of the standard. But, it could be seen that the antioxidant potential of extracts at higher concentration was comparable to that of the standard at lower concentrations. The results thus proved that the extracts were potent against ABTS. It was obvious that the RCL and RCR extracts showed most efficient inhibition which was comparable to that of the standard, Ascorbic acid.

In accord to their scavenging activities the following order was established.

Ascorbic acid > RCL > RCR > RCS > RCF > RCP

Above results were analogous with some other authors also, Leelaprakash *et al.*, 2011 evaluated the invitro antioxidant activity in *Momordica charantia* leaves. They explained
that the methanolic extracts of leaves of *Momordica charantia* were more potent than aqueous extracts in both DPPH and ABTS methods.

**5.1.3.2 DPPH scavenging**

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is reported to be a firm free radical which is used often for assessing the scavenging ability of compounds, for estimating their antioxidant nature. This method derives its basis from the reduction reaction of methanolic DPPH radicals in solution while the availability of an antioxidant able for donating hydrogen via production of the nonradical reduced DPPH-H (Blois, 1958). Thus, the loss of its characteristic deep purple ($\lambda_{\text{max}}$ 515–517 nm) colour after accepting hydrogen from the corresponding donor to yellow acts as an indicator and is measured spectrophotometrically (Soares et al., 1997). The increased inhibition of DPPH with higher concentration of plant extracts displayed their increasing antioxidant ability.

The results observed display that RCP failed to scavenge free radicals and showed poor inhibition percentages and the highest $IC_{50}$ value. However, RCL was most efficient out of all, owing to its lowest $IC_{50}$ value, which was almost $1/3^{rd}$ of that for Ascorbic acid.

According to the various $IC_{50}$ calculated, following decreasing order of DPPH$^+$ radical scavenging ability of the extracts was inferred:

RCL > RCF > RCS > RCR > Standard > RCP

DPPH scavenging involved in vitro study by Singh et al., 2009, revealed that key phenolic compounds responsible for the antioxidant ability of leaves of *R. communis Linn.* were gallic acid, quercetin, gentisic acid, rutin, epicatechin and ellagic acid.

Zahin et al., reported in 2009 that, the methanolic root extract of *Plumbago zeylanica*, rhizome of *Acorus calamus*, stem of *Hemidesmus indicus* and bark of *Holarrhena antidysenterica* showed efficient inhibition of DPPH at a concentration of 100 µg/ml, being, 77.0% (maximum) in case of *H. indicus*, 73.41% for *P. zeylanica*, 20.88% by *A. calamus* and 20.06% in case of *H. antidysenterica*
Similar results obtained in a study by Volluri et al., 2011 indicated that methanol extract of plant *Bacopa monniera* showed powerful nitric oxide radical scavenging, reducing power and DPPH activity.

HO et al., 2012 examined the possible antioxidant activities of the methanol and water extracts of 31 medicinal wetland plants in Taiwan. They showed that *Rotala rotundifolia, Juncus effusus var. decipiens, Cyperus iria, Salix warburgii, Lindernia antipoda, Kyllinga brevifolia,* and *Typha orientalis* possessed both high antioxidant activities and high total polyphenol contents. Kumbhare et al., 2012 studied the antioxidant activity in-vitro for various extracts (petroleum ether, chloroform and methanolic) of stem bark of *Moringa oleifera* and found that the methanolic extract was good scavenger of DPPH radical.

In a study by Rao et al., 2013 the powder extracts of *R. communis L.* (Euphorbiaceae) were tested for their antioxidant activity by DPPH radical scavenging activity. An appreciable ability against the DPPH radicals was observed, where the regression line efficiently showed its antioxidant potential which was comparable to ascorbic acid.

5.1.3.3 Metal Ion Chelating

Oxyradical generation could be prevented by metal ion chelation. (Sudha et al., 2011) On chelating Fe$^{2+}$, Ferrozine are capable of forming a red color complex which can be quantified. In the presence of other chelating agents, this reaction is limited such that there is a decrease in the red color intensity. The color reduction, gives an estimate of the chelating activity. (Soler-Rivas et al., 2000) The metal ion chelating activity of the extracts increased with increasing amount of sample.

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

The decreasing order of efficiency on the basis of Metal ion scavenging assay was

RCL > Standard > RCR > RCS > RCF > RCP

These assays showed that antioxidant capacity of various plant extracts of *R. communis* were different. Free radical scavenging potential was depicted in percentages, RCL
Discussion

exhibited the highest radical scavenging activity by showing the lowest IC$_{50}$ of 185.2, 154.6 and 77.5 μg/ml for ABTS, DPPH and Metal chelating respectively. The least potential was seen in case of RCP being 802, 1490 and 589.7 respectively. While, the IC$_{50}$ value of Ascorbic acid and EDTA (reference sample) was 146.3 μg/ml, 635 μg/ml and 162.82 μg/ml in ABTS, DPPH and metal ion scavenging respectively. Exhibiting the highest antioxidant activity RCL proved that it could be the best for medicinal use.

All the hydro-ethanolic extracts of *R. communis* exhibited an impressive concentration dependent antioxidant nature. However, drawing a comparision, it was deduced that RCL most obviously exhibited a powerful antioxidant potential. While RCP displayed the weakest antioxidant ability.

Similar study was done by Leelaprakash *et al.*, 2011 evaluated the invitro antioxidant activity in *Momordica charantia* leaves. They explained that the methanolic extracts of leaves of *Momordica charantia* were more potent than aqueous extracts.

- **Enzymatic antioxidant potential**

Biological system counterpoises the overproduction of ROS by an array of enzymatic antioxidant system. Each extract displayed a similar motivational trend directly proportional to the increment in their concentration. However, these assays also showcased different antioxidant abilities of various crude extracts of the plant. The most powerful potency of SOD, CAT and GPx was seen in RCL.

5.1.3.4 SOD activity

Superoxide anions are a species produced by various enzyme cascade reactions of auto-oxidation or by non-enzymatic ETCs reducing molecular oxygen monovalently. These are basically oxygen centered radicals with selective reactivities, their reductive capability could also reduce certain iron complexes (Londonkar and Kamble, 2011). The major defense antioxidant enzyme SOD, is known to scavenge the superoxide anions by catalyzing their conversion into H$_2$O$_2$ which is less toxic (Curtiz and Mortiz, 1972). The SOD activity observes a rising trend with respect to concentration of the extracts, which suggests an increase in their antioxidant nature with increasing concentration.
From the observations it could be said that both the leaf and the stem extracts showed highest specific activities

\[ RCL > RCS > RCF > RCR > RCP \]

### 5.1.3.5 CAT activity

However, \( \text{H}_2\text{O}_2 \) is also toxic. Catalase is another antioxidant enzyme which is important for the defense mechanism against the \( \text{H}_2\text{O}_2 \) radicals. CAT functions for the breakdown of \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \) and \( \text{O}_2 \) (Scandalios, 1997), without the production of more free radicals (Ragavendran et al., 2012). The CAT activity also revealed proportional increment with the rising concentration of both the extracts.

The results verified that the leaf and the stem extracts exhibited the highest potential for CAT whereas, pods showed the lowest.

\[ RCL > RCS > RCF > RCR > RCP \]

### 5.1.3.6 GPx activity

The third category of enzymatic antioxidants studied was Peroxidase. These are oxidoreductases which utilise HO as receptor of electron for their mode of action in catalyzing different oxidative reactions, reducing HO into \( \text{H}_2\text{O} \) and thereby, oxidizing a varied group of substrates (Gache et al., 2010). Our study demonstrated that the Peroxidase activity was also concomitant to higher extract concentrations.

The highest specific activities were shown by leaf extract, followed by the stem extract, further followed by comparable activities of flowers and roots. While, that of the pods were the lowest.

The decreasing trend for GPx activity can be represented as:

\[ RCL > RCS > RCF > RCR > RCP \]

Nagavani and Rao in 2010 discussed the antioxidant activity in the *Michelia champaca* flowers. The highest activity of enzymatic-antioxidants was noticed in fresh flowers than dry flowers. The activity of SOD, CAT and GPx was seen to be higher in the aqueous
extract than the methanol and ethanol extract. They also reported about the antioxidant activity in *Nymphaea nouchali*, brum flowers.

After the evaluation of phytochemical nature, *in vitro* anti-diabetic and anti-oxidative potential of the extracts, it was evident that RCP showed an evidently exceptional trend of anti-diabetic and anti-oxidative inability as compared to all other extracts studied for instance, it displayed an extremely low α-glucosidase and α-amylase inhibition with IC$_{50}$ values as high as 988μg/ml and 805μg/ml which were approximately more than four times and twice the value of their respective Acarbose values. Thus, it was obvious that it could not be used as an anti-diabetic agent. It also showed disappointingly low free radical scavenging and enzymatic antioxidant activities and therefore could clearly not be regarded of anti-oxidative value.

Ragavendran *et al.*, in 2012 focused on evaluating the level of enzymatic and non-enzymatic antioxidant status of *Aerva lanata*. This study revealed that the quantification of enzymatic antioxidants (SOD, CAT, GPx, GST, Ascorbate oxidase, peroxidase, polyphenol oxidase) as well as non-enzymatic antioxidants (Vitamin C, GSH). Based on the finding of this study *A. lanata* was considered to have a good reservoir of enzymatic and non-enzymatic antioxidants. Its free radical scavenging ability was also assessed.

Singh *et al.*, 2012 carried out an *in vitro* anti-oxidative examination of various parts of *Stevia rebaudiana* by estimating total flavonoids, total phenols, ABTS radical scavenging, DPPH radical scavenging, SOD, CAT and peroxidase activities. Root and leaf extracts of *S. rebaudiana* at a concentration of 800 µg / ml DPPH, exhibited the highest radical scavenging activity of 82.36 % and the least of 47.05 % by the flower extract.

A study by Iqbal *et al.*, 2012 demonstrated the antioxidant and antibacterial activities of different extracts of plants *Periploca aphylla* and *R. communis*, *in vitro*. This study evidenced that, both the plants proved their antioxidant ability and can replace synthetic drugs available.
A study by Patel et al., 2012 observed OH, H₂O₂, O²⁻ scavenging activity, reducing power, chelating capacity, total antioxidant activity of *Withania somnifera* and *Aloe vera* extracts. The results showed that both the plants possessed excellent anti-oxidant and free radical scavenging abilities. Screening of both the plants at different doses (100, 150 and 200 μg / ml) helped to reveal the potential of individual plants. *Withania somnifera* showed better hydroxyl radical scavenging, reducing power and superoxide radical scavenging compared to *Aloe vera*, which possessed better chelating power than *Withania somnifera*. The results suggested that all extracts might prove be a potent sources of antioxidants that could serve as therapeutic agents in the future.

Methanolic leaf extract of *solanum surratense* was analyzed for phytochemicals, *in vitro* antioxidant and antidiabetic potentials by Manjusha et al., in 2013. It was established that the extract showed maximum inhibition opposition to α-glucosidase and α-amylase enzymes with IC₅₀ 43.66μg/ml and 98μg/ml respectively. Moreover, the screening of phytochemicals confirmed the availability of flavanoids, phenolic compounds, alkaloids, carbohydrates and absence of tannins, oils and fats.

The antioxidative bioefficacy of hydro-ethanolic extract of *R. communis* leaves was tested by evaluating its enzymatic antioxidant potential both *in vitro* and *in vivo* recently, by Verma et al., 2014. The *in vitro* tests included assay for SOD, CAT, and GPx activity. The study revealed that the hydroethanolic extract of *R. communis* leaves (RCLE) had a considerable enzymatic antioxidant potential. This was also proven *in vivo*, by treating experimental mice under oxidative stress with RCLE for a period of 45 days and estimating the antioxidant enzymes in their liver, pancreas and kidneys.

The purpose of our study was to carry out *in vivo* investigation of management of diabetes and related oxidative stress, but this extract had proved to be of poor potential in this regard. Henceforth, despite of the presence of high amount of fatty acids, moderate alkaloids, flavonoids, terpenoids and glycosides and low amount of resins and saponins in RCP, it was not selected for further *in vivo* studies. While, RCL, RCF, RCS and RCR
verified as potent anti-diabetics and antioxidants, therefore, were selected for treating Alloxan induced diabetic Swiss albino mice.

### 5.2 Evaluation and comparison of the antihyperglycemic and antidyslipidemic potential of various hydroethanolic extracts of different parts, isolated compounds from *R. communis* and Glibenclamide in alloxan-induced diabetic male adult Swiss albino mice.

DM is possibly the quickest budding metabolic turmoil in the world. It is attributed by inadequate insulin emission and/or insensate target tissues to metabolize insulin functions. In addition to primary effects, DM is characterized by increased risks like hyperglycemia, dyslipidemia, defective antioxidant defense, hypertension, decreased fibrinolytic activity, increased platelet aggression and severe atherosclerosis (Reusch, 2011). Thus there is a challenging need to discover and develop newer and appropriate therapies

Synthetic drugs, particularly Sulfonylureas, such as Glibenclamide, Glipizide, and Tolbutamide, are oral hypoglycemics widely used to encourage the release of insulin from pancreatic β-cells in type 2 diabetic individuals (Mohammed *et al.*, 2013). Amongst the above mentioned drugs, Glibenclamide is the most commonly used. It functions by restricting ATP-sensitive K⁺ channels of pancreatic β cells. This reticence, results in cell membrane depolarization and opening of voltage-gated Ca⁺ channels and thereafter, provoking an exaggerated intracellular movement of Ca⁺ into the β cells which further triggers insulin emission. (Sanofi-Winthrop, 2010). Although, presently existing drugs take an important part in the management of DM, these drugs bring with them restrictions in the form of objectionable adverse effects for instance, severe hypoglycemia, weight gain, secondary failure, and incapacity to seize pancreatic degeneration or diabetic implications which can be associated to DM induced oxidative anxiety (Erejuwa *et al.*, 2011).

Since ages, numerous plant-based compounds have been admired world over for controlling diabetic hyperglycemia and have also verified to grant indicative liberation
and help in controlling the resultant complications from the disease including OS and dyslipidemia (Jarald et al., 2008). Quite a few herbs have been confirmed to aid the restoration of β-cells and in overcoming insulin opposition.

Most of the plant parts and decoctions that have been used in indigenous systems of medicine are important sources of phytochemicals, many of which are pharmacological and therapeutic agents. One of the important factors in favor of this is their, by and large, side-effect free nature as compared to modern allopathic drugs. The need for search of newer plant based anti-diabetic and anti-oxidant drugs continues (Grover et al., 2002)

Alloxan has been utilized to promote experimental DM because of selective damage of the insulin-generating β-islets of pancreas. This drug instigates a multiphasic vascular glucose reaction when injected to an experimental model. These reactions are analogous to contrary alterations in the plasma insulin level and then chronological ultra structural β cell alterations, finally leading to necrotic cell loss. A number of experimental investigations have shown that soon after alloxan administration it provokes an abrupt increase in the oozing out of insulin, in the availability or non-availability of glucose. This specific alloxan-induced insulin emission takes place for a limited period followed by the total censorship of the islet reaction to glucose even if glucose is present in high concentrations. Moreover, the function of alloxan in the pancreatic tissue is preceded by its swift uptake by β cells, was observed to be one of the major attributes in determination of alloxan diabetogenicity. Further, the reduction reactions in the β cells undergo in the availability of various reducing agents like GSH, cysteine, ascorbate and protein-bound sulphydryl (-SH) groups. Alloxan reacts with two -SH groups at the sugar attaching site of glucokinase following the creation of the disulfide bond and rendering the enzyme inactive. Consequential of alloxan reduction, dialuric acid is produced which is then flip re-oxidized to alloxan, thus maintaining a redox loop for the over-production of ROS and superoxide radicals. The O$_2^-$, free Fe$^{3+}$ from Ferritin and convert them into Fe$^{2+}$ and Fe$^{3+}$. In association, O$_2^-$ radicals undertake dismutation to yield H$_2$O$_2$ via the action of SOD. Consequently, highly reactive OH$^-$ are generated by the Fenton reaction in the availability of Fe$^{2+}$ and H$_2$O$_2$. It has been known to affect the DNA of islet cells of pancreas (Mohammed et al., 2013). The disintegration of β cell DNA occurs in alloxan exposure, which instigates poly ADP-ribosylation, a process involved in DNA repair. Antioxidants
enzymes like SOD, CAT and the non enzymatic scavengers of hydroxyl radicals are known to control alloxan toxicity (Rohilla and Ali, 2012)

5.2.1 Effect on morphology of experimental animals

A marked reduction in the body weight was observed subsequent to alloxan injection, which however was normalized to a certain extent after the 45 day treatment with glibenclamide and RCLT, RCFT, RCST, C1T and C4T. This alleviation of reduced body weights depicted the improved normalcy of the physiological condition of experimental animals due to the treatments under study. RCRT showed an exceptional decrease in the body weights. The order of the most potent treatment in restoring the body weight to the least potent treatment could be presented as:

C1T>RCLT> C4T>GT> RCST> RCFT>RCRT

The body weight restoring capacity of the treatments studied was in consistence with several previous studies, viz., substantial weight loss of 24.6% was observed by Gokce and Haznedaroglu (2008) post alloxan treatment. However, the treatment with the aqueous extract of *Posidonia oceanica* leaves at the dose of 150 and 250 mgkg\(^{-1}\)BW recovered the weight loss in 15 days but the difference in the initial and final body weight was found to be insignificant.

The reduced body weight of alloxan induced diabetic swiss albino mice was also reported to be maintained after 21 days administration of methanol extracts of *Stevia rebaudiana* roots and leaves given at 300mg/kg BW. (Singh *et al.*, 2013; Singh and Garg 2014)

Tomar and Sisodia, 2014 evaluated *Annona squamosa* Linn hydroalcoholic extract for its antidiabetic potential as compared to Glibenclamide in alloxan-diabetic rats and concluded that the extract led to significant elevation in the body weights of the diabetic animals at a dose of 350 mg/kgBW and 700 mg/kgBW comparable to that by glibenclamide at 5mg/kg for 28 days.

Sebai *et al.*, 2015 assessed the shielding effect of *Rosmarinus officinalis* essential oils (ROEO) and *Lavandula stoechas* essential oils (LSEO) opposite to reproductive damage
and OS in alloxan-diabetic male rats. They deduced that ROEO and LSEO prevented the alloxan-induced body weight decrease.

### 5.2.2 Effect on various biochemical parameters of alloxan induced diabetic male swiss albino mice

#### 5.2.2.1 Effect on Fasting Blood Glucose/ Hyperglycemia:

Subsequent to the alloxan administration there was marked increase in the blood glucose level. This owed to the occurrence of several definite toxins that lead to the enormous demolition of the pancreatic β cells, stimulating a condition of the basic deficiency of hormone insulin without disturbing other sorts of islets and thus rendering a hyperglycemic state. Herbal extracts may prove beneficial in treating the ailments caused by the DM administration, as they act by stimulating glucose utilization by peripheral tissues post alloxan action.

In the current investigation it was recorded that all the five treatments under observation including that of glibenclamide (10 mg/ kg BW) and the four crude extracts of various parts of *R. communis* (300 mg/ kg BW) showed potent anti-hyperglycemic activities, with glibenclamide showing 53.8% decrease, RCLT displayed a glucose concentration lowering of 51.65%, C1T by 43.57%, RCFT by 36.6%, RCST showcased a decrement of 33.54% and that of RCRT was hypoglycemic by causing a decrease by 61.09% in the elevated glucose level.

Thus the decreasing order of anti-hyperglycemic nature of the treatments could be:

RCRT > GT > RCLT > C1T > C4T > RCFT > RCST

Kesari, 2005 studied the antidiabetic activity in aqueous extract of *Murraya Koenigi* leaves, common in Indian cooking. He observed that various doses of the extract could lower the blood glucose level in alloxan induced rabbits. Habib *et al.*, 2005 compared the effects of gliclazide with neem leaf, *Catharanthus roseus* leaves and bitter melon fruit juice extract significantly lowered the glycemic level with maximum reduction of 45% observed in case of treatment with gliclazide. Eddouks *et al.*, 2005 studied the hypoglycemic status of
Discussion

*Lepidium sativum* seeds aqueous extract in streptozotocin induced diabetic rats. They observed that, acute and chronic (15 days) oral treatments caused significant reduction of blood glucose level. After this, Somani *et al.*, 2006 evaluated the antidiabetic activity in *Butea monospema* flowers. Similarly, *Pongamia pinnata* flowers also have been reported by Punitha *et al.*, (2006) to have antihyperglycemic value.

The observed hypoglycemic nature of RCRT was in consistence with a previous study by Shokeen *et al.*, who reported in 2008, that 50% ethanolic extracts of *R. communis* roots at a dose of 500mg/ kg BW for a period of 20 days had hypoglycemic effect. According to the study, the highest hypoglycemic effect was constantly seen at the 8th hour up to which the study had been undertaken.

Moreover, aqueous and methanolic flower extracts of *Punica granatum* also have antidiabetic activity had been reported by Bagri *et al.*, 2009. Then, Ghosh *et al.*, in the year 2008 investigated the antidiabetic and antioxidative effect of water extracts of *Psoralea corylifolia* (somraji) and *Trigonella foenum-graecum* (methi) seeds in differential and amalgamated manner in streptozotocin-diabetic male rats. They concluded that, the amalgamated extract of above plants had higher potent antidiabetogenic activity than different extracts. Sabu and Kuttan (2009) studied the antidiabetic and antioxidative potential in *Terminalia belerica* Roxb. In their investigation, continous administration of methanol extract of *T.belerica* fruits in water was evaluated in rats with alloxan induced diabetes for 9 days. The treatment was observed to significantly lower the serum glycemic level of treated rats to 54% as compared to normal.

Zulfiker *et al.*, 2010 studied the antidiabetic and antioxidative activity in *Scorparia duleis* in alloxan induced albino mice. The oral treatment of plant extract at a dose of 100 & 200 mg/kg BW was given to fasting, glucose loaded (200 mg/kg BW) mice, with regard to normal control during 3 hrs study period and in alloxan-caused (150 mg/kg body weight) diabetic mice as compared to Metformin (600μg/kg BW) during 2 weeks study period. Then, Chakraborty and Das, 2010 evaluated the antihyperglycemic activity in *Cinnamomum tamala* leaves on the glycemic level of diabetic rats for three weeks.
Adminstration of leaf extract at 250mg/kg BW for three weeks significantly reduced the glycemic level.

Shukla et al., 2011 evaluated and compared the antidiabetic effects *Caesalpinia bouncucella* and *Stevia rebaudiana* ethanolic extracts in alloxan-diabetic experimental rats. They concluded in their results that the oral treatment of both extracts at 300 and 400 mg/kg BW showed reduction in glycemic levels significantly. *C. bonducella* seeds showed more potent antidiabetic potential as compared to *S. rebaudiana* leaves. Ragini et al., also investigated in the same year, the antidiabetic activity in *Shorea tumbuggaia* Rox. Alcoholic leaf extract of the plant at the higher doses of 400 and 800 mg/kg BW significantly reduced the glycemic concentration. Antidiabetic activity of *S. trumbuggaia* might have been due to the encouragement of surviving β cells.

Tomar and Sisodia, 2014 evaluated *Annona squamosa* Linn hydroalcoholic extract for its antidiabetic potential as compared to Glibenclamide in alloxan-diabetic rats and concluded that the extract led to significant elevation in the body weights of the diabetic animals, at a dose of 350 mg/kgBW and 700 mg/kg BW comparable to that by glibenclamide at 5mg/kg for 28 days

Furthermore, studies on antihyperglycemic nature of various plants had been carried out which were found analogous to this study. Sung-Hsun et al., 2015 hypothesized that cordycepin from *Cordyceps militaris* normalized blood sugar levels and improved the indicators of diabetes in alloxan-induced diabetic mice model. Different doses of cordycepin (8, 24, and 72 mg/k BW) were intraperitoneally administered to diabetic mice for 21 days daily. Acute toxicity test on normal mice was carried out by giving them maximum tolerance dose of cordycepin (3600 mg/kg BW) daily. A 47% reduction of the blood glucose level and significant improvement of oral glucose tolerance were noticed after the effective dose of cordycepin was administered.
5.2.2.2 Effect on Serum total protein

During DM, there is an increased protein catabolism and the resultant amino acids (the catabolic product) feed gluconeogensis and accelerate ureagenesis, resulting in hyperurenenemia. Moreover, the decrease in the serum total protein post alloxan administration was overcome by treatment with insulin, which aggravates the transport of amino acids through the cells and stimulates the protein manufacturing process (Gyton and Hall 2000).

Hydro-ethanolic extracts of all the four crude extracts, isolated compounds and glibenclamamide significantly increased the level of serum total proteins in alloxan induced diabetic mice. This indicated that, various treatments were proved effective in deleting the negative effects on protein synthesis caused by the alloxan administration.

The order in which the various treatments studied caused total serum protein normalization was:

RCLT> C1T> RCST> RCFT=C4T> GT> RCRT

Singh et al., 2013; Singh and Garg 2014 reported the serum total protein restoring effect of methanolic extracts of Stevia rebaudiana roots and leaves respectively when given at 300mg/kg BW for a duration of 21 days to alloxan-diabetic swiss albino mice.

5.2.2.3 Effect on lipid profile

Diabetic dyslipidemia is characterized by the presence of increased plasma TG concentration, lowered HDL cholesterol concentration and heightened LDL-cholesterol amount (Mooradian, 2009). The major reason of the alterations in lipid profile linked with diabetic dyslipidemia is the exaggerated free fatty-acid (FFA) emission from insulin-resistant fat cells (Taskinen, 2003; Krauss and Siri, 2004; Solano and Goldberg, 2005; Chahil and Ginsberg, 2006). The higher unrest of FFAs into the liver while, the availability of sufficient glycogen reserves motivates TG production, which further promotes the emission of apolipoprotein B (ApoB) and VLDL cholesterol. The higher amount of VLDL cholesterol particles and plasma TG levels, lead to reduction in the level of HDL cholesterol as well as increment in the LDL- cholesterol particles.
The restricted insulin emission results in heightened metabolism of lipids from the adipose tissue and their release into the plasma. Many different aberrations in metabolic and regulatory mechanisms, due to insulin deficiency, are the reason for the observed gathering of lipids. HDL is said to have characteristic anti-atherogenic abilities and it is well known that an increment in HDL shares an indirect relation with CHD, while, a decrement portends the cardiovascular risk. Hyper-cholesterolemia, is also known to have a positive correlation with CVD (including atherosclerosis, myocardial infarction and cerebral paralysis), largely depending on the oxidation of LDL, the main cholesterol carrier in plasma. LDL takes the cholesterol from liver to all tissues whereas, HDL facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism (Taskinen, 2003; Krauss and Siri, 2004; Solano and Goldberg, 2005; Chahil and Ginsberg, 2006)

Comparison of effect of glibenclamide, the hydro-ethanolic extracts and isolated compounds from *R. communis* leaves proved efficient to certain extent in restoring the variated levels of lipid profile parameters after alloxan induction. The decreasing order of anti-dyslipidemic behavior on the basis of the restoration of total serum cholesterol values can be recorded as:

RCRT> C1T> RCLT > RCST> GT> RCFT> C4T

Rajasekaran *et al.*, 2006 reported that the quietened plasma levels of HDL and aggravated plasma levels of LDL, VLDL in diabetic rats were almost normalized after oral administration of *Aloe vera* leaf gel extract.

Abd-Elraheem *et al.*, 2009 evaluated the consumption of ginger extract in alloxan induced-diabetic rats and their lipid profile. They reported TC, TG, LDL depreciated post-treatment.

Ahmed *et al.*, 2010 reported that the methanolic whole plant extract of *Vinca rosea* at highest dose (500mg/kg BW) demonstrated significant anti-hyperglycemic activity than that at lower dose (300mg/kg BW) in diabetic rats. The improvement was seen in parameters like body weight and lipid profile as well as regeneration of β-cells was
observed. Then, Dangi and Mishra, 2010 studied the hypolipidemic effect of *Capparis aphylla* stems in streptozotocin-diabetic rats. The report mentioned, significantly decreased glycemic levels in normal and diabetic rats (p<0.01) during oral glucose tolerance test with single oral dosing of 300mg/kg BW of extract and 30mg/kg BW of active fraction; significantly (p<0.01) quietened TC, TG, VLDL and LDL levels; and significantly (p<0.01) promoted serum HDL after 7 days oral treatment with active fraction.

Devi *et al.*, 2011 reported that the levels of TC, TG, LDL, HDL, and VLDL were found to be reduced significantly (P<0.001) after the treatment with *Aegle marmelos* leaf extract in streptozotocin (stz) induced diabetic male albino rats, when compared to that of diabetic control rats. Similarly, Asad *et al.*, (2011) reported that the administration of *Acacia nilotica* leaves extract showed hypoglycemic and anti-platelet aggregation activity in diabetic rats as compared to that of glyburide. Ragini *et al.*, 2011 studied antihyperglycemic and hypolipidemic in ethanolic extracts from plant *Shorea tumbuggaia* Roxb. in alloxan-induced diabetic rats. Treatment with plant extract led to a significant (p<0.05) decrement in the heightened TG, TC, LDL, VLDL in 21 days; a significant (p<0.05) increase in HDL levels in 28 days. Five week oral administration of aqueous extract of *Momordica charantia* showed significant dip in TC (21% P<0.01), TG (20% P<0.01), LDL (20% P<0.01) and increase in HDL (45% P<0.05) (Bano *et al.*, 2011). Also, Volpato *et al.*, 2011 evaluated the effect of *Morus nigra* aqueous extract treatment on lipid profile from diabetic and non-diabetic rats.

Aljamal *et al.*, 2012 worked on the effect of Rosemary (*Rosmarinus officinalis*) on lipid profile of diabetic rats and noted that the diseased rats showcased a rise in the sugar, TC, TG and LDL levels and a dip in the level of HDL. But the administration of the plant regulated the changed levels by causing a decrease of 20% in sugar level, 22% TC, 24% TG, 27% LDL, and increase 18% in HDL. Eze *et al.*, 2012 experimented on the potency of ethanolic leaf extract of *Mucuna pruriens* (fabaceae) on lipid status in alloxan-diabetic rats and reported significant reduction (p<0.05) in the serum TC, TG, LDL-C levels and increased HDL-C concentration in alloxan-induced diabetic treated groups.
Singh et al., 2013; Singh and Garg, 2014 discussed the antidyslipidemic properties of methanolic leaves and roots extracts of Stevia rebaudiana at 300mg/kg BW for 21 days in alloxan induced diabetic swiss albino mice.

Recently a study by Sharma et al., (2014) reported that dyslipidemia in DM is may be due to the extreme transportation of fat reserves from adipose tissues because of low usage of glucose. An obvious increment was observed in the level of serum TC, TG, LDL, and VLDL-Cholesterol whereas the level of HDL is significantly (P<0.05) depleted in DC, as compared to that in NC. 45 days treatment of Pithecellobium dulce pod extract (hydroethanolic) caused a significant (P<0.05) reduction in TC, TG, LDL and VLDL with simultaneous increase in HDL-Cholesterol, as compared to diabetic control mice. Tomar and Sisodia, 2014 evaluated Annona squamosa Linn hydroalcoholic extract for its antidiabetic potential as compared to Glibenclamide in alloxan-diabetic rats and concluded that the extract at a dose of 350 mg/kgBW and 700 mg/kgBW led to significant reduction in serum lipid profiles like TC and TG; but significant increase in serum HDL level in diabetic rats compared to untreated group.

5.2.2.4 Effect on hepatic glycogen content

Glycogen, glucose reserve, is a direct measurement of the functioning of insulin in the body. Insulin secretion stimulates the carbohydrate intake being stored in the form of glycogen in liver and skeletal muscles by a coordinated action of two enzymes i.e., glycogen synthase and glycogen phosphorylase (Taylor et al., 1996). Defects in the process of insulin, is because of the lack of glycogen synthetase activating systems (Annamala and Augusti, 1980) and/or risen functioning of glycogen phosphorylase (Abdel- moneim, 1997), is resulted in a pinpointed dip in the amounts of insulin which could be a major reason for the postprandial hyperglycemia.

The depletion in the hepatic glycogen content subsequent to the alloxan administration might be owing to the desruption of β cells caused by alloxan (Wilson and Leiter, 1990). It is relevant that glycogen amount in the liver decreases as it is dependent on insulin for influx of glucose.
From the results it was evident that all the treatments studied were capable of not only restoring but improving the total hepatic glycogen content. However, the decreasing order of their hepatic glycogen restoring activity can be enlisted as:

GT > RCFT > RCLT > RCST > C1T > RCRT > C4T

El-Shenawy and Abdel-Nabi in 2006 examined the hypoglycemic outcome on normoglycemic and in alloxan-diabetic mice after treatment with ethanol extract of *C. droserifolia* leaves. In their study, they found out that 0.31 g/kg BW dose of the extract significantly decreased the hepatic glycogen content by 56.6% in diabetic controls as compared to non-diabetic animals, while, significantly increased it by 48.2% as compared to diabetic group.

Sharma and Garg (2012) investigated the hepatic glycogen level in hydroethanolic bark extract of *Butea monosperma*. Post alloxan induction at the dose of 150mg.kg BW significantly reduced the level of hepatic glycogen .But the subsequent treatment of bark extract for 45 days significantly increased the level of glycogen in liver by 77.7%.

Singh and Garg 2014, recorded the improvement in hepatic glycogen content of alloxan diabetic mice after the administration of roots of *Stevia rebaudiana* in methanolic extract form at 300mg/kg BW for 21 days. Treatment with various extracts of *S. rebaudiana* and stevioside studied in alloxan diabetic mice by Singh and Garg, 2013, for 21 days showcased a significant rise in the hepatic glycogen content. Among all leaf, flower and root extract resulted in almost equal increment in the hepatic glycogen content. These results confirmed to an extent its insulin impending effect, which is also comparable with that of glibenclamide.

Recently, Sung-Hsun *et al.*, 2015 reported a 214% increase of hepatic glycogen content in alloxan-induced diabetic mouse model by giving them maximum tolerance oral dose of cordycepin from *Cordyceps militaris* at different concentrations of (8, 24, and 72 mg/kg BW) daily for 21 days.
5.2.2.5 Effect on Tissue Total Protein content:
Diabetic condition leads to hypoproteinemia i.e., the decrease in serum total proteins. Moreover, the amino acid intake or protein synthesis in the liver has been reported to be suppressed due to hepatic disorders (El-Shenawy and Abdel Nabi, 2006). As per the results discussed earlier, all the treatments studied exhibited a positive effect on decreased tissue total protein content in DM. The leaf extract was the most efficient in restoring the protein content in liver and kidney, while, C1 proved to be the most efficient in case of pancreas. However, following common trend of enhancement of lowered protein content can be established: RCLT > RCST > C1T > C4T > RCRT > RCFT > GT

The methanolic bark extract of *Tectona grandis* Linn. showed potent antioxidant and antidiabetic in alloxan induced diabetic rats and it can be used for the drug development (Ananthi *et al*., 2003).

Chitra *et al*., 2010, also explained the antidiabetic and free radical scavenging potential of the seed extract of Strychnos nuxyomica. They reported the significant increase in SOD, CAT and total protein level and decrease in LPo, TC, serum Creatinine and blood urea nitrogen level in alloxan induced diabetic rats, which distinctively showed the antioxidant property of the extract.

Rajaram, 2013 evaluated the *in vivo* antioxidant and antidiabetic activity of the methanolic bark extract of *T. grandis* Linn and reviewed that protein synthesis is decreased in all tissues due to absolute or relative deficiency of insulin in alloxan induced diabetic rats.

5.3 *In vivo* comparative analysis of various treatments, normal and diabetic control groups
Free radicals and ROS are usually vital for essence of life, as they participate in cell signaling cascades and are utilized by phagocytes for their bactericidal action. In amalgamation with these set and essential functions, ROS are also created in all the respiring organisms as a result of mitochondrial respiration, which involves O$_2$ in the course of production of ATP, by the pairing of ETC and OS. OS has potential adverse effects on cells and thus, ROS are employed in the etiology and onset of several disorders including DM (Dasgupta *et al*., 2012). It is currently well established that O$_2$ is inevitable
for life, it possesses the potential of becoming toxic, very rarely and concludes in the production of highly violent agents like ROS. The massive reactivity of ROS may instigate the body to play a host for a variety of disorders, consequential tissue desruption and necrosis in several cases (Varier et al., 2010). The current study also showcased visible changes in antioxidant status and necrosis in the atopsy sections of liver, pancreas and kidney of experimental diabetics.

5.3.1 Antioxidant potential of various hydroethanolic extracts, isolated compounds from *R. communis* and Glibenclamide

5.3.1.1 Effect on lipid peroxidation:
During the diabetic state, hypoinsulinemia augments the action of fatty acyl coenzyme, an oxidase that marks the β-oxidation of fatty acids, ensuing in LPo. Heightened LPo concludes in production of toxic, free radicals, which renders the membrane function impaired due to depreciating levels of membrane linked enzymes and receptors. This further, results into cell damage and complications such as atherosclerosis, brain and kidney injury. Diabetic models treated with plant extracts displayed pinpointed decrement in LPo. This might owe to the scavenging action of the plant drug (Geetha et al., 2011).

LPo is one of the key attributes of chronic DM, and LPo caused tissue injury has been reported in diabetic state (Feillet-Coudray et al., 1999). Hyperglycemia produces ROS, which further leads to LPo and in turn, cause membrane damage (Hunt et al., 1988). Pavana et al., 2007 registered the incidence of motivated TBARS amounts and reduced antioxidants in the erythrocytes of diabetic rats. They also described that risen biomembrane-LPo processes could be the motive underlying this, which concludes in the changes in the antioxidant status. Heightened TBARS in the tissues of the diabetics hint the unnecessary production of free radicals, commencement of LPo mechanism and malfunction of antioxidant defense machinery which restricts the generation of excessive free radicals (Resmi et al., 2006). Exaggerated TBARS in diabetic animals indicate that peroxidative damage may perhaps be associated in the progression of diabetic maladies (Brown and Goodman, 1998; Andreassi, 2003; Hasanain and Mooradian, 2004).
Free radicals could be generated through the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radicals created may react with these polyunsaturated fatty acids in cell membranes leading to LPo and conclude in a rise in synthesis of more free radicals. The elevated LPo in the diabetics may be owing to the considerable increment noticed in the amount of TBARS and lipid peroxidative markers-hydroperoxides in the liver and kidney of diabetic rats (Stanely et al., 2001).

There is an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha and Pari 2003, Ananthan et al., 2003), which can be correlated to the fact that, the tissues include rather higher amount of simply peroxidizable fatty acids. During DM, liver showed a comparatively harsh mutilation of antioxidant ability than kidney. The kidneys exhibit a typical trend of alterations in DM (Aragno et al., 2000). The increased oxygen free radicals could be chiefly as a result of elevation in blood glycemic level, which on auto-oxidation produces free radicals and secondly because of the properties of diabetic drug, alloxan (Szkudelski, 2001).

In the present study, the various treatments played a commendable role in moving the heightened LPo level towards normalcy in all the organs. The standard drug could somehow not normalize the lipid peroxidation status in liver and pancreas while it almost restored the value in kidney. The trend of lipid peroxidation potential in liver can be listed as:

RCLT> C4T> RCFT> C1T> RCST> RCRT> GT

Can et al., 2004 examined the Aloe vera pulp and gel extract. They found that these extracts have hepatoprotective effect against the damage caused by DM. The LPo was decreased after the treatment.

Sathishsekar and Subramaniam, 2005 suggested that the Momordica Charantia (bitter gourd) seed extract imparted speedy preventive effects against LPo by scavenging of free radicals and thus, lowering the risk of diabetic complications in streptozotocin induced diabetic rats.
Chung et al., 2006 demonstrated that the TBARS concentration in all diabetic groups was elevated as compared to that in normal groups. *Rhus verniciflua*, *Sophora japonica*, and *Paeonia suffruticosa* extract treated diabetic rats exhibited reduced concentrations of TBARS in the blood than control diabetic rats, which displayed higher TBARS level than non-diabetic controls, after 4 weeks of streptozotocin injection. *Citrus sinensis* and *Punica granatum* peel extract administration at 25 mg/kg BW of *Citrus sinensis* or 200 mg/kg of *Punica granatum* was found to normalize all the adverse changes induced by alloxan, revealing the antidiabetic and antiperoxidative potential of test fruit peel extracts. (Parmar and Kar 2007).

Rajkumar et al., 2011 analyzed the antioxidant ability of gallic acid (GA) on membrane LPo and osmotic fragility in alloxan-induced diabetic Wistar rats. GA was given orally at 5, 10, and 20 mg/kg BW for 45 days, following this liver and kidney were investigated for the degree of LPo. The results revealed that GA prevented the uprightness of erythrocyte membrane in diabetic rats as exhibited by lesser percentage of hemolysis and restriction of H$_2$O$_2$ induced peroxidation. The anti-hyperglycemic activity of GA in alloxan-induced diabetic rats was similar to that of standard drug, glibenclamide.

Geetha et al., 2011 stated that leaf extract of *Achyranthes rubrofusca* at 200mg/kg/ BW/ day for 28 days resulted in the marked dip in the enhanced TBARS concentration.

Dangi and Mishra 2011, have observed that *Capparis aphylla* stem extract imparted quick preventive properties against LPo by scavenging the free radicals and thus lowering the risk of diabetic complications. They explained that, *C. aphylla* stem extract administration might efficiently modify the pancreatic cell impairment and antioxidant status towards normalcy.

Sirovina et al., 2013 compared the properties of flavonoids quercetin and chrysin on LPo and histopathological alterations in liver of diabetic alloxan induced mice and their potential to chelate Fe$^{2+}$ ions *in vitro*. After two days of alloxan induction, flavonoid preparations (50 mg kg$^{-1}$ per day) were administered intraperitoneally for 7 days. The LPo was estimated by calculating the MDA generation using the TBARS test.
5.3.1.2 Effect on antioxidant enzyme markers

Maladies during DM are characteristic of various factors like glucose auto-oxidation resulting into free radical production, cellular oxidation/reduction imbalances and reduction of antioxidative abilty. Free radicals perform a vital part in pathogenesis of several serious disorders, besides DM, such as cancer, liver cirrhosis, CVD, atherosclerosis etc. Biological system counterpoised the unavoidable creation of ROS by choice of enzymatic defense mechanism. These enzymatic antioxidants SOD, CAT, GPx, play a key part in cell wall defense against the LPo and other cellular injury. SOD saves the cell wall by catalyzing $O_2^-$ into $H_2O$ and $H_2O_2$. CAT functions at high concentration of $H_2O_2$, and it voluntarily detoxifies it into $H_2O$ and $O_2$. GPx is responsible for the catalysis of breakdown by oxidizing glutathione with the generation of its conjugates. SOD, CAT and GPx are enzymes that demolish the peroxides and play a significant part in imparting antioxidant defences to an organism. When the homeostasis between ROS synthesis and antioxidant defence is lost, it concludes into OS, which via a chain of proceedings downregulates the cellular actions resulting into various pathological states (Bandhopadhyay et al., 1999). The enzymatic antioxidants i.e., SOD, CAT and GPx form the first line of defense mechanism to protect the organism from ROS mediated oxidative damage (Nonaka and Manabe, 1991). The damage caused by the OS is likely to be elevated after the damage caused to antioxidant enzymes themselves and renders inactive due to the glycation-induced OS, finally leading to the perturbation of cellular redox condition (Shin et al., 2006).

SOD has improved β cell tolerance in the oxidative anxiety induced DM. Lowering of SOD action may be underlying the insufficient activities of CAT and SOD observed which may lead to elevated production of $H_2O_2$ and $O_2$. Extract administered rats showcased higher activity of SOD and CAT protecting the free radical buildup in liver and pancreas. Prevention of $O_2^-$ and $H_2O_2$ by SOD and CAT may ameliorate alloxan toxicity (Geetha et al., 2011).

GPx, an enzyme including selenium, and GST work in accord with glutathione (GSH) in the degradation of $H_2O_2$ (or) several other organic hydroperoxides to non-poisonous products at the cost of the GSH. Lowered activities of GPx may deduce from radical instigated inactivation and glycation of the enzyme. Further, presence of inadequate GSH
might as well deprive the activity of GPx. The decremented action of GST seen in diabetic condition may be owing to the inactivation led by ROS.

GSH is an important non-protein thiol present in living organisms which functions in synchronizing the body’s antioxidant defense procedure. Amidst every non-enzymatic antioxidant defense systems, non-protein thiols are amongst the prime defenses that neutralize the OS. GSH takes an important part in several cellular activities. GSH has been noted for destruction ROS and free radicals produced during metabolic processes. Besides, it even prevents membrane lipids and aids in movement of proteins through membranes (Kangralkar, 2010). GSH occurs in βcells of pancreatic islets and prevents the membrane from OS by regulating the redox condition of proteins inside the membrane (Inove et al., 1987)

**Effect on SOD after 45 days**

The herbal extracts and isolated compounds proved more capable in recovering the depleted SOD activity as compared to Glibenclamide in liver and kidney to a great extent. Whereas, in pancreas, GT exhibited successful enhancement of SOD activity, but, only after RCLT.

In liver:

C1T > RCLT > RCFT > RCST > RCRT > GT > C4T

In kidney:

C4T > RCLT > RCST > C1T > RCRT > RCFT > GT

In pancreas:

RCLT > GT > RCFT > C1T > RCST > C4T > RCRT

**Effect on CAT after 45 days**

From the table 3.1.3 it is evident that the treatments benefit the CAT activity in the following order:

RCLT > C1T > RCST > RCFT > C4T > RCRT > GT

**Effect on GPx after 45 days**

The calculated values give the following general order of GPx activity enhancement:
The exceptions being in the case of kidneys where the order of efficiency was noted as:
C1T > RCLT > RCST = RCFT > RCRT > GT > C4T

**Effect on GSH after 45 days**

In the investigation under consideration, administration of test groups with various herbal preparations concluded in an appreciable increase in the amount of GSH in comparison to untreated ones, thus revealing the protection offered by these herbal preparations in combating oxidative stress due to diabetes. RCLT proved to be most potent in alleviating the lowered amount of reduced GSH. Elevation in the GSH content is mainly owing to the availability of antioxidant in different extracts and isolated compounds. As the lipid peroxides are converted into alcohol and not to Malondialdehyde (MDA) in the presence of GSH. Thus, the increased presence of GSH resulted into decrease in the MDA level. The trend on GSH lowering observed in liver and pancreas was

RCFT > RCLT > C1T > RCST > C4T > GT > RCRT

Kaleem et al., 2006 evaluated the antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. They observed that streptozotocin resulted in decline in the antioxidant activity but further the extract enhanced the decreased activity of SOD, CAT and GSH-Px in all treated rats. Before this, Sathishsekar and Subramanian (2005) observed a significant elevation in the activity of hepatic and renal SOD, CAT and GSH-Px of Streptozotocin induced diabetic rats after the oral treatment of 150 mg/kg BW of aqueous extract of *Momordica charantia* for 30 days. Similarly, Manonmani et al., 2005 observed that the hydroethanolic extract of *Cassia fistula* flowers at the dose of 10mg/kg BW, when administered orally for 15 days, significantly lowered the cardiac SOD, CAT and GSH-Px in alloxan induced diabetic rats.

Oral administration of diabetic rats with *Allium sativum, Azadirachta indica, Momordica charantia*, and *Ocimum sanctum* extracts at 500 mg/kg BW not only lowered the blood glycemic concentration on the other hand even inhibited the generation of lipid peroxides, reactivated the antioxidant enzymes, and restored levels of GSH and metals in the streptozotocin induced diabetic rats (Chandra et al., 2008). Bhandari and Ansari in 2008
observed in their work that aqueous extract of *Embelia ribes* Burm at the dose of 100 and 200 mg/kgBW significantly elevated the reduced level of GSH, SOD in pancreatic tissues of streptozotocin-diabetic rats.

Bhutkar and Bhise, 2011 reported the antioxidative property of ethanolic extract of *Tmarindus indica* bark in alloxan induced diabetic rats. They observed that, GSH and glycogen level had displayed a significant decrement after induction of DM with Streptozotocin, these parameters were known to be elevated in the hepatic tissue with extract treatment. Aqueous extract of *Scoparia dulcis* at doses of 250 mg/kg BW/twice a day respectively for a period of 3 weeks significantly elevated these parameters in hepatic tissue of rats (Das and chakraborty, 2011). After 8 weeks oral administration of *Withania somnifera* root and leaf extract to the diabetic rats, the decreased SOD, CAT, GPx and GSH levels showed significant increase. Proving that *W.somnifera* root and leaf extract have vital antioxidant activity.(Udaykumar *et al.*, 2010). Sadiq *et al.*, 2010 found out that at 500mg/kg BW, the stem extract of *Tinospora cordifolia* reduces the free radical levels in diabetic rats in comparison to normal rats. Stem extract of *T. cordifolia* normalizes the decreased levels of antioxidants SOD, GPx and CAT in liver tissues. Chitra *et al.*, 2010, also explained the antidiabetic and free radical scavenging potential of the seed extract of Strychnos nuxyomica. They reported the significant increase in SOD, CAT and total protein level and decrease in LPo, TC, serum Creatinine and blood urea nitrogen level in alloxan induced diabetic rats, which distinctively showed the antioxidant property of the extract.

Lukiati *et al.*, 2012, researched in order to expose SOD status of pancreas of diabetic rats and the protective properties of *Curcuma heyneana* ethanolic extract. They investigated that the extract was capable to elevate SOD activity and fix the pancreatic β cells harm on DM rats induced by MLD-STZ at 72 mg/kgBW. In an attempt to investigate the hypoglycemic, hypolipidemic and antioxidant properties of a blend of curcumin from *Curcuma longa*, linn, and partially purified product from *Abroma augusta*, linn. in streptozotocin induced DM. Hussain, 2012 reported that there was an increase in GSH, SOD and CAT which displayed the antioxidant potential of the mixture. He indicated that these alterations initially countered the OS in DM. Thus, a gradual depreciation of the antioxidative method may be among the factors which could results in chronic DM.

Kannadhasan *et al.*, 2012 concluded that there was more significant increase (P<0.001) in the level of antioxidants like SOD, CAT and GSH in polyherbal hydroethanolic extract (PHHE) treated groups as compared to that of diabetic control. They also established that
all the crude herbal extracts and extracts of its isolated or purified compounds possessed the antioxidant potential.

Ramesh et al., 2012 investigated *Commiphora mukul* gum resin for its antioxidant property and LPo lowering potential in pancreas and heart of streptozotocin induced diabetic rats. They observed that after the streptozotocin administration, the antioxidant enzyme activities were significantly lowered in the pancreas and heart in comparison to the control group. But the treatment of ethanolic extract (200 mg/kg BW) to diabetic rats for 60 days significantly reverted the values above towards normalcy.

Verma et al., 2014 evaluated the antioxidant and anti-hyperglycemic potential of *R. communis* flower extracts at 300mg/kg BW on alloxan induced swiss albino mice. It was found that the extract was capable of significantly controlling the SOD, CAT and GPx levels. Sharma et al., in the same year carried out the in-vivo evaluation of antihyperglycemic, antidyslipidemic and antioxidant activities of hydro-ethanolic pod extract of the plant *Pithecellobium dulce* in alloxan induced diabetic swiss albino male mice. The findings indicated significant anti-hyperglycemic, antidyslipidemic and antioxidant activity of *P. dulce* pods. The activity of enzymatic and non-enzymatic antioxidants was normalized to such an extent that helped in reduction of oxidative injury in the tissues of diabetic animals.

Sebai et al., 2015 analyzed the shielding effect of *Rosmarinus officinalis* essential oils (ROEO) and *Lavandula stoechas* essential oils (LSEO) in opposition to reproductive damage and OS in alloxan-diabetic male rats and they deduced that ROEO and LSEO prevented the alloxan-diabetes induced OS. They displayed an elevation MDA and H$_2$O$_2$ concentration along with depletion of sulphydryl group and SOD, CAT, GPx after alloxan induction. Their results indicated that ROEO and LSEO imparted a preventive aid against alloxan-induced reproductive function damage and OS in male rat.

Sharma et al., 2015 evaluated the powder of seeds of plant *Trigonella foenum-graecum* L. in alloxan diabetic rats for its antioxidative nature and antihyperglycemic potential. They showed increased production of hydrogen peroxide, increased accumulation of malondialdehyde (MDA) and 4-hydroxynonanal (4HNE) and thus immense OS in tissues on one hand, and reduction in SOD), GPx and CAT activities on the other. However, they
also showcased the improved levels of $\text{H}_2\text{O}_2$, MDA, and $4\text{HNE}$ and SOD, GPx, and CAT activities as well as transcription of these genes in liver and the brain of diabetic rats treated with $T. \text{graecum}$ for 15 days.

**3.1.3 Effect on albumin and globulin**

The results reveal that the post alloxan decline in the serum albumin and globulin contents is significantly increased after Glibenclamide treatment. However more efficient recovery of the normal levels is done by various extracts of $R. \text{communis}$. Thus, the order of descent in restoration of serum albumin and globulin is:

RCFT $>$ RCLT $=$ C4T $>$ RCST $>$GT $>$C1T $>$RCRT

The reduction in total protein and albumin may be due to microproteinuria and albuminuria, which are imperative clinical indicators of DN (Mauer et al., 1981) and/or might be due to risen protein catabolism. It has been deduced that insulin provokes the merging of amino acids into proteins (Almdal and Vilstrup, 1988).

Kaleem et al., 2008 investigated the oral treatment of $A. \text{squamosa}$ (300 mg/kg BW) aqueous extract, for activities of insulin, C-peptide, albumin, albumin/globulin for 30 days, in diabetic rats and reported the significant increase in the activities of all marker enzymes near to control levels.

In an investigation by Devaki et al., 2011, potential of of $\text{Bauhinia tomentosa}$ L. aqueous extract on alloxan induced type 2 DM wistar albino rats was studied. They observed a sharp fall in total protein and a slight decline in albumin, globulin and a significant change in A/G ratio were observed in diabetic rats. The total protein and albumin were decreased in diabetic rats, but increased with $B. \text{tomentosa}$ treatment. They reported that, in treated groups, $B. \text{tomentosa}$ improved the insulin secretion and reversed the altered protein profile by exerting the protein sparing effect.

Kemsari et al., 2011., in their work on the antihyperglycemic activity of $\text{Mangifera indica}$ Linn. in alloxan diabetic rats, observed reduction in plasma total protein, albumin and globulin in alloxan induced rats. They explained that, the lessening amount of protein may
be due to microproteinuria and albuminuria (Makare 2001; Tuvelmo 1997; Bakris 1997) and / or may be due to increased protein catabolism (Almdal and Vinstrup, 1988).

Hosseini-Zijoud et al., 2012 reported the daily oral administration (for 30 days) of Persian shallot extract at 200mg/BW on some biochemical parameters in streptozotocin-induced diabetic rats. The plasma TC, urea, protein and albumin were significantly increased in extract treated groups.

5.3.2 Histopathological changes by the hydro-ethanolic crude extract and isolated compounds from R. communis leaves and Glibenclamide treatment.

Histopathological studies confirm the changes occurred in tissues such as liver, kidney and pancreas after diabetes, and treatment of induced diabetes with the most efficient extract of R. communis plant and standard drug glibenclamide.

5.3.2.1 Liver

Liver is the largest organ having a huge mass of glandular tissue in the body. It is situated beneath the diaphragm in the upper abdomen. Liver stands clearly in the trail of blood vessels that communicate substances from the digestive tubes, as liver receives its major supply of blood from the hepatic portal vein, which carries venous blood from the digestive tube, pancreas and spleen. Along with the digested and absorbed materials that are assimilated and stored in the liver, the portal blood also carries various toxic materials, which are detoxicated in or excreted by the liver. Under low power examination, the liver is seen to be composed of the masses of epithelial, parenchymal cell (hepatocytes) arranged in anatomizing and branching plates that form a three dimensional structure. Between these plates, sinusoidal blood spaces are present. Portal areas or portal canals are the areas lying in the small amount of connective tissues, comprising branches of the portal vein, hepatic artery, the bile duct with lymphatic vessel. These portal areas (afferent vessels) are arranged so as to form a delineate structure of the liver tissue.

Diminished glycogen content in liver cell was also determined by several scientists (El-Shenawy and Abdel-Nabi, 2006). However no change in liver tissue was observed in the control mice. These symptoms lead to necrosis of the cell in DM induced experimental
mice. It may be due to defective activation of glycogen synthase and insulin deficiency in mice. Hepatic fat accumulation is a well-recognized complication of DM with a reported frequency of 40–70%.

Sirovina et al., 2013 compared the effects of flavonoids quercetin and chrysin on LPo and histopathological alterations in liver of diabetics and their capability to chelate Fe^{2+} ions in vitro. They hypothesized that the treatment of diabetic mice with flavonoid solutions results in decreased number of vacuolated cells and degree of vacuolization of the liver tissue.

Recently, Aboonabi et al., in the year 2014, studied the histopathological changes in diabetic rats. They revealed that the destruction of the liver architecture of the hepatocytes in the diabetic group showed the signs of necrosis, degeneration, dilatation, and inflammation in the central vein and blood vessels. Then in the same year, histopathological assessment of the macerated *Allium sativum* (garlic) on cytoarchitectural alterations in alloxan (150mg/kg BW) induced diabetic rat liver was studied by Oyebadejo et al. The results revealed preservation of cellular architecture, reappearance of hyperplastic hepatocytes, cellular restoration, vascular dilatation and pyknotic nuclei in treated group as complete regeneration when compared to non-diabetic and diabetic control group that showed focal area of necrosis, vascular congestion, hyperplasia, vacuolation, inflammation and cellular degeneration.

Alloxan induced mice exhibit significant alterations in structure of liver cells. Dilation in sinusoids, infiltration of portal triad, ballooning degeneration of hepatocytes, granular cytoplasm degeneration and neutrophilic infiltration was observed in liver cells (Shanmugasundarm et al., 1983; Leegwates et al., 1984; Ghosh et al., 2001; Thakran et al., 2004).

The hepatic lobule comprises of several portal canals at its periphery and in the centre is the central vein (efferent vessels) which is the tributary of the inferior vena cava, from which the parenchymal cells radiate out. This explains that the blood flow is from the
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periiphery to the sinusoidal channels between the plates of the liver to the central vein (Leeson and Leeson, 1981).

Moreover, the deformities of hepatocytes in DM were recovered by treatment with leaf extract of R. communis. A unique feature of glycogen infiltration was observed after the treatment. The accumulation of glycogen was specifically observed only in treatment with leaves extract of R. communis after diabetes induction. This may be due to increase in insulin content which converts glucose into glycogen in liver cells. The treatment of DM with standard drug, glibenclamide shows normalization of liver cells. The crude extract also exerted an effect on DM induced in liver cells in significant correction.

5.3.2.2 Pancreas

Pancreas is a large elongated organ, lying in the concavity of the duodenum and extending behind the peritoneum of the posterior abdominal wall, toward the left to reach the hilum of the spleen. Pancreas is both an exocrine and endocrine organ. The endocrine portion of the pancreas, the islet of langerhans, is scattered throughout the pancreas as irregular, spheroidal masses of palestaining cells with a rich vascular supply. In the islets of Langerhans, cells are arranged in irregular cord, between which are the capillaries. Granules are not seen in ordinary hematoxylin and eosin preparations, as these are soluble in alcohol and would be washed out. Special staining methods demonstrate the three main types of the cells namely the α (alpha), β (Beta) and Δ (delta), and a few nongranulated clear (C) cells. In the islets, β cells are centrally located and usually numerous (about 70% of the islets cell population), whereas, α and Δ lie peripherally and are fewer in number (20 and 5% respectively). And the number of these cells varies from cell to cell. The β cells contain numerous granules characterized by a crystalloid core of polygonal shape, the crystalloid probably being insulin, with species difference being recognized by the shape of these crystals. Each cell type secretes a different hormone. The β cell produces insulin, which acts on the cell membrane (particularly liver and muscle) to facilitate glucose transport into the cell, with a subsequent lowering of the blood sugar level. The β cell actually synthesizes preproinsulin, which is converted to proinsulin by splitting off a 23 amino acid residue from the C terminal backbone, thus allowing it to fold back on itself and connect via disulphide bond. The proinsulin consists
of 35 amino acid which is cleaved off in golgi apparatus and immature vesicles, producing insulin and C peptide. The insulin consists of an A chain (21 amino acids) and a B chain (30 amino acids) coupled by two disulphide linkages in the form of a zinc complex. Pancreas of control mice shows normal islets of langerhans. Induction of alloxan at 150 mg kg\(^{-1}\) BW resulted in DM.

The alloxan induction damages the β cell of langerhans which is reported to diminish the level of insulin, resulting in enhanced glucose content and reduction in the glycogen content in body. This is in agreement with earlier reports (Ghosh et al., and 2001Gholamali et al., 2005). The regenerative effect in pancreas cells was observed after the treatment of crude extracts of different crude extracts and isolated compounds from the plant (\textit{R. communis}) and glibenclamide. Deformation resulted due to DM shows recovery after the treatment of different extracts.

Aqueous extract of \textit{Annona muricata} leaves when given orally at the dose of 100 mg kg\(^{-1}\) BW for 30 days led to remarkable improvement of the islets of langerhans of diabetic rats. An interesting striking feature observed in the glibenclamide treated group 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).

Treatment with various extracts in diabetic rats showed pancreatic islet regeneration. These results are in agreement with Sharma and Garg (2012). They reported correction of DM after the treatment with different extracts of \textit{Butea Monosperma}.

Histopathological examination done by Alimohammadi et al., in 2013 after treatment of \textit{Nigella sativa} (NS) on diabetic (streptozotocin induced) rats showcased that NS at 5 mg/kg B.W. partly improved hepatic glycogen content and prevented a major amount of the pancreatic β cells. The concentration of islets, cells and islets diameter were brought into being statistically on comparison to the control group (p<0.01).

Tomar and Sisodia, 2014 evaluated the hydroalcoholic extract of \textit{Annona squamosa} Linn for treating alloxan- diabetic rat model. They investigated that 28 days treatment of 350
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mg/kg BW and 700 mg/kg BW of the extract and 5mg/kg BW of standard drug, glibenclamide on significant histological changes caused by alloxan in the pancreas of diabetic rats. Treatment with A. squamosa extract however showed partial regeneration of β cells in pancreas.

5.3.2.3 Kidney

Kidneys are big, bean shaped organs cited on both lateral sides of the spinal column in the retroperitoneal tissue of the abdominal cavity. Each kidney is wrapped in a lean fibroconnective tissue capsule called renal fascia. On the upper pole of each kidney lies an adrenal gland. The median border of the kidney is concave containing a deep fissure, the hilum, from which the renal sinus extends into the substances of the kidney. All the blood vessels, excretory ducts and the ureter enter or leave through the hilus of the kidney. Within the hilus the upper part of the ureter is expanded as pelvis, is divided in the form of small and large cups known as major and minor calyces. The remaining space is filled with the aerolar adipose connective tissue. Each minor calyx envelops a conical protrusion of the renal substance called a papilla. The apex of each papilla is perforated by the openings of 10-25 collecting ducts called as area cribrosa. The nephron is the functional and tubular unit of the kidney. Each kidney contains about one to two million of nephrons, which are long epithelium lined tubes. Nephron contains several segments of different structure and different functions. The first part of the nephron, is the renal corpuscle (of Malpighi) located in the cortex, containing two parts. The proximal end of the nephron is blind and dilated into a thin-walled, cup like expansion called Bowman’s capsule, which is invaginated by a globular tuft of capillaries called the glomerulus. Both renal corpuscles have a respective vascular pole, where the efferent and the afferent enter or leave the glomerulus, and the urinary pole, where the slit like cavity of Bowman’s capsule goes into the lumen of the subsequent segment of the nephron- the proximal tubule, which continues into the Loop of Henle that in turn, is continuous with the distal convoluted tubule. The remaining part of the nephron, between them forms a Loop of Henle that is present in a medullary ray and extends for a changeable distance into the medulla.
Reports on kidney autopsy records in alloxan instigated diabetic rats exhibiting marked tubular damage; hemorrhage in the bowman’s space owing to glomerular injury etc have been stated by Ragavan and Krishnakumari (2006).

The severity of DM associated polyuria is also known to strengthen at a dose of alloxan above 140 mg kg$^{-1}$ BW (Chougale et al., 2007).

Sharma and Garg’s investigation on *Butea monosperma* in 2012, elucidated glomerulosclerosis in kidney of diabetic individuals. There was no change in proximal convulated tubule and distal convulated tubule in kidney. Alloxan at the dose below 140 mg kg$^{-1}$ BW is known to cause no damage to kidney. However, at a dose above this, stress and necrotic changes become evident.

Hypoglycemic and tissue-preventive effects of the *Persea Americana* seed aqueous extract on albino rats with Alloxan-induced DM were studied in 2013, by Ezejiofor et al. The results indicated that the extract had a significant hypoglycemic effect ($P < 0.05$) and was able to restore the histopathological injury caused due to alloxan-induced DM, as compared to the effects of glibenclamide. The seeds of *P. americana* were stated to have anti-diabetic and protective effects on some rat tissues such as the pancreas, kidneys, and liver.

Aboonabi et al., 2014 observed shrinkage and lesion in Bowman’s capsule in the kidney of diabetic rats. Pomegranate acted as an antioxidant thereby preventing oxidative damage in the diabetic kidney by improving the observed symptoms.

Findings by Leegwates et al., 1984, suggested a primary and a secondary consequence of the diabetic condition on the kidney of rats. The primary being, the DM aspect was related with hyperglycemia and was accountable for dilatation of proximal and distal tubules inside the cortex. The secondary effect, also called the individual response aspect, was related with inflammatory processes. A short connecting segment (the arched collecting tubules) passes to a straight collecting tubule and, this radially ends into papillary duct that ultimately opens into a minor calyx via an area cribrosa (Leeson et al., 1981).

In the present study, diabetic kidney exhibited shrinking of the glomerulus along with arteriolar thickening in nephrons compared to the normal control group. The extract of *R. communis* normalized the disrupted diabetic kidney cells. The glibenclamide treatment of
diabetic mice also exhibited normalization in kidney tissues. From the above histopathological study of the liver, pancreas and kidney of diabetic Control mice, it is obvious that alloxan administration severely depreciated the histology of the tissues. However, the different treatments refurbished almost all the observed deformities in pancreas, liver and kidney. Thus, can be concluded that, the treatment could cure the damage completely and giving fruitful results.

Keeping in consideration all afore mentioned results, it can be concluded that *R. communis* Leaf extract possesses significant antihyperglycemic, antidyslipidemic and antioxidative properties followed by *R. communis* flower and *R. communis* stem extracts.

### 5.4 C1 the most potent compound

The two isolated compounds collected, C1 and C4 were administered orally at a dose of 50mg/kg BW to alloxan induced diabetic mice, for controlling their diabetes and related oxidative stress; and it was found that C1 verified as the most efficient isolated compound *in vivo*.

As mentioned in the results earlier, the phytochemical analysis of C1 exhibited the presence of only alkaloids. The tests used for confirming the presence of alkaloids depend on their nature to precipitate as salts of organic acids or heavy metal (like Hg, Au, Pt, etc) compounds. For instance, in the presence of Dragendorff reagent (Potassium-bismuth-iodide solution), alkaloids turn into a reddish-brown to black-grey precipitate (Shekhar, 2008) which proves their presence.

C1 or Indole acetic acid as confirmed by characterization studies, the most efficient proved treatment regarding anti-hyperglycemic and anti-oxidative abilities, isolated from the hydro-ethanolic crude extract of *R. communis* leaves was analyzed using various spectrophotometric techniques.

As already mentioned in the results, the IR spectra 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. The current examination was in total agreement with the research investigations suggested by Krystyana and Cohen, (1986)
wherein, an amide conjugate of IAA was isolated and purified from \textit{P. vulgaris}. Results by Varalakshmi and Malliga, 2012 exposed the occurrence of IAA in cyanobacterium \textit{Oscillatoria annae}, the study had been applied to field trials for improvising the crop yield.

The NMR spectra revealed that the isolated compound C1 belongs to the class of organic compounds known as organoheterocyclic compounds. C1 contained 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 revealed a molecular ion peak at 175.1 m/z; predicting it to be Indole acetic acid.

In particular IAA is a constituent of the group of phytohormones known as auxins. IAA is normally well thought-out to be the major significant native auxin. This compound belongs to the class of organic compounds known as indole-3-acetic acid derivatives. These are compounds containing an acetic acid (or a derivative) linked to the C3 carbon atom of an indole.

Phytochemically classified as a type of Alkaloid, called as the Protoalkaloids, IAA is a derivative of tryptophan. It is established that IAA is formed by enzymatic auto-oxidation of of indole-3 acetaldehyde derived from Tryptophan via indole-3 pyruvic acid or tryptamine. Between these two routes, the main route is thought to be indole-3 pyruvic acid. Protoalkaloids possess a comparatively simple chemical structure, are formed in very few steps from amino acids and may serve as biosynthetic precursors of true alkaloids (Funayama and Cordell, 2014).

The term, ‘Alkaloids’ means alkali-like and thus, some of their behavioral characteristics are similar to naturally occurring amine complexes. These also include Proto-alkaloids and Pseudoalkaloids. Alkaloids are the organic products of natural or synthetic sources, are basic in nature and contain one or more than one nitrogen compound, which occurs heterocyclic form, and possess particular physiologically important affect on humans, when consumed in minute quantities. While, Proto-alkaloids or amino-alkaloids are those in which nitrogen is not present in heterocyclic ring (Shekhar, 2008).
Hall et al., 1975 identified the IAA in phloem and root pressure saps of *R. communis* L. by gas-liquid chromatography and mass spectrometry, IAA was identified in the phloem sap and root pressure sap of *Ricinus communis* L.

Kang et al., 1985 wrote about alkaloids and flavonoids isolated from the plant, Indole acetic acid being one of them.

Plant cells manufacture IAA from amino acid, Tryptophan (Mashiguchi et al., 2011 and Won et al., 2011). IAA has many different effects, as all auxins do, such as inducing cell elongation and cell division; plant growth and development. On a larger scale, IAA serves as signaling molecule necessary for development of plant organs and coordination of growth. IAA and a few derivatives are able to be oxidised by Horseradish peroxidase (HRP) to cytotoxic species. IAA is only toxic after oxidative decarboxylation; the effect of IAA/HRP is thought to be due in part to the formation of methylene-oxindole, which may conjugate with DNA bases and protein thiols. IAA/HRP could be used as the basis for targeted cancer, a potential new role for plant auxins in cancer therapy.

Dnyaneshwar et al., 2011 established that the antinociceptive potential of the methanol extract of *R. communis* leaves (MRCL) in mice, which was tested by acetic acid induced writhing test, formalin induced paw licking and tail immersion method after administration of MRCL at different doses of 100, 125 and 150 mg/kg BW, was due to the presence of saponin, steroids and alkaloids (like IAA) in it.

Rana et al., 2012 reviewed the plant *R. communis* as a potent antioxidant, anti-implantation, anti-inflammatory, antidiabetic, central analgesic, antitumour, larvicidal & adult emergence inhibition, antinociceptive and antiasthmatic agent. Indole-3-acetic acid was found to be one of the phytoconstituents responsible for these medicinal attributes.

5.5 Genetic expression analysis:

During DM, an altered oxidative metabolism is a result of one of the persistent exposure to hyperglycemia or of the absolute or relative insulin crisis. Insulin regulates many
reactions occupied in oxido-reductive metabolism or may be merely associative rather than being fundamental in DM. OS as estimated by indices of LPo and protein-oxidation has been displayed to be elevated in both IIDD, and NIDDM, in obese diabetic patients and even in diabetic patients without complications.

Major ROS-scavenging, antioxidant enzymes toil in rhythm to maintain an intracellular stable-condition that comprises of the SOD that dismutates $O_2^{-}$ to $H_2O_2$ followed by the synchronized activity of a set of enzymes viz., CAT, GPx that reduce $H_2O_2$ (Mittler et al., 2004). Glucose glycates CuZnSOD in the erythrocytes, reducing its activity; this may be responsible for the hindered SOD activity seen in the blood of several diabetic individuals. Significantly lessened SOD activity in obese and obese-diabetic individual’s blood has been exhibited versus healthy individuals, none of the evaluated patients or controls being anemic. Both CuZnSOD and Ceruloplasmin can part after glycation to free pro-oxidant copper ions. Levels of methylglyoxal created from intermediates of glycolysis are increased in DM, and nowadays it has been stated that the risen level of methylglyoxal is linked-up with a pitable level of glutathione in DM. Secondly, hyperglycemia results in overflow of the products of the polypol pathway along with reduction in the reduced NADPH, which is an important reducing equivalent for the regeneration of GSH by glutathione reductase (GR) (Alexandra and Max, 2013). CATs are sole agents in degrading $H_2O_2$ with none other reductant, therefore, imparting the cell an energy proficient mechanism. CATs are inevitable, accountable for the full exclusion of intracellular $H_2O_2$ produced (Scandalios et al., 1997). CATs are discretionary to substitutive $H_2O_2$-scavengers with a massive turnover rate but instead lower affinity for $H_2O_2$. Thus, GPXs prevent cell membranes from peroxidative injury, maintaining cellular uprightness. GPXs are seen in the majority of subcellular compartments and are included in the response to abiotic and biotic stresses by performing as common peroxide scavengers (Navrot et al., 2007). Fresh facts have revealed that few GPXs might as well be concerned in redox transduction while OS (Miao et al., 2006).

Free radicals produced by an incomplete reduction of $O_2$, facade a severe vulnerability not just to tissues, vital organs, membrane lipids and connective tissues but even to the nucleic acids of the cells (Edwards, 1996). ROS are extremely reactive and poisonous
depending on their ability to react randomly with roughly all biomolecules instigating negative protein aberrations, DNA strand disintegrates, purine oxidations, protein-DNA crosslinks and β-oxidation of lipids (Breusegem and Dat 2006). Thus, oxidative homeostasis is reliable on the progression of competent enzymatic and non-enzymatic ROS-scavenging pathways, called as antioxidant machinery. The havoc displayed by DM related OS has already been observed at the lipids, proteins and enzymatic levels in the previous objectives.

Up-and-coming facts suggest that generation of ROS and commencement of redox-dependent signaling cascades participate in the regulation of the antioxidant genes, which further affect the intracellular level of ROS and may give a feedback control of the ROS-reliable biological systems. Several genes included in defense, signal transduction, transcription, metabolism and cell structure are identified exposing an extremely active and surplus network of ROS-generating and ROS-scavenging genes. Antioxidant genes are vital players in this network and their expression has intense consequence in modifying ROS amounts and cellular-redox homeostasis. ROS, unlikely can potentially regulate the amount of antioxidant gene expression by imparting a feedback regulation apparatus of ROS, which is a grave constituent in the intonation of signaling cascades. Thus, parallel measurements of enzyme activity along with the antioxidant enzyme mRNA expression was necessary to deduce a correlation analyses as reports strongly suggest that the antioxidant enzyme functions of the tissues are majorly defined by the amount of their respective mRNA. (Photini, 2011)

This objective examined the data revealing the deviation in the expression of aforementioned antioxidant enzyme genes which acts as a signal resulting in the regulation of cellular redox balance/imbalance. The genetic expression was analyzed by comparing the fold change variations in the mRNA by relative quantification method using RT PCR. The relative quantification or proportional quantification estimates the mutual alteration in mRNA fold change in stable condition of a gene amid various samples and expresses it as compared to the fold change of another mRNA. It therefore, does not need a calibration curve or standards with recognized concentrations and the reference can be whichever transcript, given that its sequence is well established (Bustin, 2002).
Thus, relative quantification of mRNA of the various antioxidant enzyme genes in liver, kidney and pancreas of normal, diabetic, diabetic treated with Glibenclamid and diabetic treated with leaf extract mice was done. The values for mRNA fold change in pancreatic samples was found to be too low, were thus not included.

The degree to which a variety of tissues include antioxidative enzymes may be a main determinant for their unitized susceptibility to cellular injury. To evaluate the scope to which pancreatic β cells enclose antioxidant enzymes, Markus et al., 1997 estimated the expression and regulation of SOD, CAT, and GPx. They described the scarcity of proficient H\textsubscript{2}O\textsubscript{2}-inactivating enzyme apparatus in β-cells for the exemplary sensitivity of β-cells to toxic injury in progression of autoimmune or chemical DM. Thus, dissimilarly, various tissues and insulin-synthesizing cells actually fail to adjust their amount of antioxidant enzyme expression in reaction to these characteristic conditions of OS. There is no information available for the aptitude of different stress inducers and poisonous compounds to increase CAT or Gpx expression in insulin-generating cells to a certain extent. Merely Mn SOD has been registered to illustrate a partial rise. The expression of H\textsubscript{2}O\textsubscript{2} - inactivating enzymes CAT and cytoplasmic Gpx was maximum in liver and kidney; lesser in spleen, lung, adipose tissue, heart muscle and adrenal gland; and very less in skeletal muscle, brain, intestine, and pituitary gland. The minimum expression levels, mostly less than 10% of that in the liver, were detected in pancreatic islets.

Talking about the present study, the results exhibited before, observed that, the maximum abundance of expression of SOD and GPx genes was highest in the calibrator sample, normal kidney. The expression of CAT gene was however more in normal liver, while that of GSR gene was equal in both the normal samples of kidney and liver tissues. This organ-specific variation observed in mRNA expression can be due to the respective cytotoxic damage. The fact that all the genes showed maximum expression in normal untreated samples implies that the natural antioxidant system comprises of various antioxidant compounds and numerous antioxidant enzymes such as SOD, CAT, GPX and GSR.
Alloxan induced injury to \( \beta \)-cells resulted in excessive generation of ROS and scarcity of insulin. Hyperglycemia and LPo were generated as a consequence of crisis of insulin and massive level of ROS, respectively. O\(^2-\) radicals, H\(_2\)O\(_2\) and OH\(^-\) radicals are the majority of ROS which lead to nucleic acid, proteins and other macromolecules-damage, and transformations that buildup DM (Saglam et al., 2012) and subsequent diabetic complications. In normal conditions of oxidative homeostasis, O\(^2-\) radicals are converted to H\(_2\)O\(_2\) by SOD. Then, H\(_2\)O\(_2\) is further, converted into molecular O\(_2\) and H\(_2\)O by one of the CAT or GPx. Furthermore, GPx can decrease lipid peroxides and various organic hydroperoxides which may be extremely poisonous to cells. Thus, SOD, CAT and GPx comprise the standard constituents of the antioxidant system and their deficits can result into OS. In addition, GSH conjugated to xenobiotic compounds through the help of GSR.

But, the diabetic, untreated condition led to the action of the antioxidant enzymes, SOD, GPx, GSR and CAT to reduce in liver, kidney tissues of mice, as talked about earlier. Likely, reports were observed for the mRNA expressions of SOD, GPx, GSR and CAT genes in liver and kidney were significantly poorer in diabetic than in normal tissues. These findings indicate that the action of antioxidant enzymes like Cu-Zn SOD, CAT, and GPx were linked to the expressions of their respective gene. Earlier investigations deduced that the activities and mRNA expressions of antioxidant enzymes, Cu-Zn SOD and CAT, were reduced (Sadi et al., 2008) and those of GPx were increased (Sadi and Guray, 2009) in the liver tissue of rats, after 2-3 weeks of induction of DM. While, after 5-6 weeks of DM induction, the rat liver tissue exhibited an increment in Cu-Zn SOD mRNA expression combined with a rise in SOD activity, and an appraisal in CAT gene expression as compared to a reduction in enzyme activity in the renal cortex (Matsunami et al., 2010)

These findings verified that OS alters the antioxidant defense machinery in the various organs in their own explicit pattern. The mRNA expressions of antioxidant enzymes in diabetic animals and humans have been inadequately worked on, besides, the up regulation/ down regulation of mRNA expressions of antioxidant enzymes in the study. In that opinion, report fetched from clinical and animal investigations indicated that diabetic patients and animals embrace significant reduced antioxidant enzymes, and risen
The observed aberrations in the mRNA expressions of antioxidant enzymes in untreated DM could be due to oxidation of transcriptional factors responsible for the initiation machinery of antioxidant enzymes transcription process (Matsunami et al., 2010).

Glibenclamide is amongst the excessive recurrently prescribed oral hypoglycemic drug available (Nathan et al., 2009). It instigates insulin release as well as hinders hepatic glucose generation consequential in reduction of blood glucose level (Rendell, 2004). Nonetheless, the usage of glibenclamide is restricted due to its causative prolonged hypoglycemia, high secondary failure rate and other adverse events (Mukai et al., 2007; Harrower, 1994). In comparison to the earlier mentioned findings it can be concluded that, in spite of its hypoglycemic effect, Glibenclamide failed to ameliorate OS in pancreatic tissues of diabetic rats. Accountably, glycemic or metabolic memory might have been the reason underlying the incapacity of the drug to attenuate enzymatic antioxidant condition and protect the tissues from lipid peroxidative injury in diabetic pancreas despite of its commendable hypoglycemic effect (Erejuva et al., 2011).

It was interestingly pragmatic that, the treatment by leaf extract of *R. communis* brought symptomatic increase in the expression of various genes. Such that the mRNA level of SOD and GSR genes was almost restored or elevated but was not normalized; that of GPx was completely restored, in fact was more than that in normal in case of liver. The mRNA level in case of CAT gene also displayed a motivational trend. The results further revealed that, the treatment with Glibenclamide provided no relief to the OS condition, as it could not incorporate the increase in mRNA fold change in most of the genes. However, it caused a significant mild increase in CAT expression in both kidney and liver and also very slightly raised the GSR expression in kidney but insignificantly.

Studies with respect to the consequences of OS on antioxidant enzyme activities were significantly diverse from no changes (Rajpathak et al., 2009), decrease (Hussain et al., 2012) and increase (Sabry and Bahr, 2013; Limaye et al., 2003) owing to the duration of the experiments, the age of animals or different potencies of the treatments investigated. A variety of evidences with similar, contradictory and no changes; that suggest impaired
genetic antioxidant condition is observed in OS linked up with DM are discussed respectively:

Millward et al., 2002 investigated the regulation of antioxidant genes in type 1 diabetes patients with diabetic nephropathy, in response to hyperglycemia. They recorded, that the patients had higher level of CAT and GPX mRNA in normoglycemic individuals with respect to the patients with nephropathy, under normal glycemic conditions versus the patients without complications, under hyperglycemic versus normoglycemic conditions. Our present findings were in agreement with their findings.

Pallavi et al., 2003 also researched on the outcome of hyperglycemia on antioxidant enzymes by studying the enzyme activities and genetic expression of CAT, SOD and GPx, renal cortex of rats after 6 weeks of streptozotocin-induced diabetes. LPo and protein oxidation in the renal cortical homogenate were first undertaken to verify the condition of OS. RT-PCR analysis was then put to use to confirm if stable condition of transcription levels were altered in diabetics. The enzyme assays exhibited a difference in significant changes in activities of CAT, SOD and GPx as compared to an opposite trend of their gene expressions. An appraisal in GPx and Cu-Zn SOD mRNA accompanied the increase in respective enzyme activities. However, increase in expression of the CAT gene was contrary to the decreasing enzyme activity, suggesting some post-translational modification

Sindhu et al., 2004 demonstrated the activities and protein expression of enzymatic antioxidants (SOD, CAT and GPx) in streptozotocin-diabetic and the results of insulin and therapeutic antioxidants univalently and in amalgamation in male Sprague-Dawley rats. Vitamin E and C were the antioxidant-treatment along with Insulin. The animals were noticed for a duration of 4 weeks. Diabetic animals exhibited remarkable weight reduction, lowered activities of Cu Zn SOD and CAT and normal GPX activity. Along with this, the expression of all antioxidant proteins also saw a reduction in the diabetic rats in contrast to the controls. Therapeutic Insulin controlled weight loss and moved the enzyme activities and their protein expression to normalcy. The study found that the amalgamation of insulin and antioxidants restored all measured antioxidant enzyme
protein expressions and activities. Thus, they registered that diabetes-related alterations in antioxidant status could be ameliorated by Insulin and/or antioxidant therapy.

A different type of study was undertaken by Flekac et al., 2008, who observed the gene polymorphisms of SOD and CAT in DM. They established that lowered action of scavenging enzymes was seen in patients with DM. It was hypothesized that the preventive action of antioxidant scavenging enzymes might have declined due to OS. This investigation was studied the linked action of gene polymorphisms of certain scavenger enzymes and the diabetic complications.

In a research led by Sadi and Guray, 2009 studied the gene expression, protein expression and the activities of MnSOD GPx consequential of the streptozotocin-induced diabetes. MnSOD mRNA and protein expressions saw no change. The GPx mRNA expression also did not see any change but its protein analysis displayed an increase. Treatment with Lipoic acid (LA) a water- and lipid-soluble antioxidant, led to a significant reduction in the elevated MnSOD protein expression and resulted in significantly risen GPx activities in diabetics. The RT-PCR and protein analysis deduced the observed increase in activity was not regulated at the gene level, as both mRNA and protein levels did not change. Another treatment with vitamin C, a powerful water-soluble antioxidant, however led to an increment in MnSOD genetic expression while the protein expression and the enzyme activity failed to change statistically. Dissimilar response was seen in case of GPx where, enzyme activity raised significantly which was possibly due to post-translational modifications as mRNA and protein expressions did not experience any alteration. These conclusions along with their earlier elucidations on CAT and Cu-Zn SOD suggested the being of a network of control mechanisms, antioxidant enzyme regulation while their protective action against the OS damage.

In 2010, Matsunami et al., applied Hyperbaric Oxygen (HBO) exposure to model system for investigating oxidative stress. They investigated the result of HBO revelation on the gene expression of cytosolic SOD (Cu-Zn SOD), cytosolic GPx (GPx-1), and CAT genes of the liver, skeletal muscle, and pancreas of streptozotocin-induced DM rats, with the aid of RT PCR. The mRNA expressions of Cu-Zn SOD and CAT genes decreased significantly (p < 0.001), while that of GPx rose significantly (p < 0.001). This trend of gene expressions was common to all the studied organs of rats. The mRNA levels further
decreased on HBO exposure in comparison to those from DM-induced rats not exposed to HBO. Accordingly, the activities of these enzymes changed in synchronization with the mRNA levels.

Saglam et al., 2012 reported the determination of cinnamon and sugar tea extract for protective effects on streptozotocin (STZ)+nicotinamide-induced diabetic rats. The therapeutic potential of cinnamon and tea extract on DM was assessed through analysis of their effects on both oxidative stress and DNA damage. Cinnamon and sugar tea extracts were administered to diabetic treatment groups for 30 days after induction of DM. At the end of the experiment, the levels of GPx, SOD, CAT and MDA were determined in the liver homogenate. Comet assay was put to use to assess DNA damage in blood cells. DNA damage as assessed by comet length was increased in diabetic groups and decreased in cinnamon and sugar tea treated groups relative to controls. Their study revealed that the treatment with either cinnamon or sugar tea extract had protective effects against OS in type 2 diabetic rats. Nemoto et al., 2012 investigated alteration in mRNA expression of hepatic glutathione peroxidase (GPx) and superoxide dismutase (SOD) in streptozotocin-induced diabetic mice by berberine and observed that the level of SOD and GPx was decreased in diabetic mice which is recovered by the treatment of berberine.

Jelena et al., 2013 elucidated the associative effect of hyperglycemia lowering and antioxidant activities of α-lipoic acid (LA) add to its effectiveness in protecting against kidney damage and various diabetic complications. The accompanying rise in MnSOD activity was found to be linked to upregulated gene expression.

Ahmed et al., 2013 investigated the influence of walnuts diet on streptozotocin-induced diabetic mice and observed that in case of DC group the expression of GPx was overexpressed and expression of SOD and CAT was found reduced. Treatment of walnet managed the mRNA expression of SOD and CAT and reduced the expression of GPx.

Sabry and Bahr, 2013 studied the regulation of the abilities and genetic expression of antioxidant enzymes, SOD, CAT, GPX, GST, β-cell CLL/lymphoma 2 (Bcl-2) and insulin akin to growth factor-1 (IGF-1) in streptozotocin induced diabetic rats by their treatment with Curcumin. The treated group was administered with curcumin orally at 15 mg/5 ml/kg BW for 6 weeks. Diabetic rats displayed significant rise in blood glucose, TBARS and activities of all antioxidant enzymes only significant lowering of GSH as
compared to the control group was noted. Gene expression of Bcl2, SOD, CAT, GPX and GST raise significantly in case of diabetic untreated rats in contrast to the control group. The treatment with curcumin to diabetic rats significantly moved their blood sugar and TBARS values towards normalization while increasing antioxidant enzymes activities and GSH. Along with this, curcumin treated rats displayed significant rise in gene expression of IGF-1, Bcl2, SOD and GST as compared to the non diabetic and diabetic untreated rats. The findings finally concluded Curcumin as an antidiabetic agent, induced hypoglycemia by up-regulation of IGF-1 gene and reduced the diabetes induced OS by elevating the GSH, activities and gene expression of antioxidant enzymes and Bcl2.

Deprem and Gulmez, 2014 examined the gene expression level of glutathione peroxidase 1 (GPx1) in the liver of healthy and diabetic mice, its localization in the tissue, and structural changes in the liver. In this study, 36 Swiss albino mice were divided into 3 groups: experimental (diabetic) (n = 15), sham (n = 15), and control (n = 6). The GPx1 enzyme activity of the experimental group was lower compared to the sham and control groups (P < 0.05). There was no difference between the experimental and sham groups in terms of the gene expression of GPx1; however, a statistically insignificant decrease was observed in the experimental group compared to the sham group.

Sharma et al., 2015 evaluated the powder of seeds of plant *Trigonella foenum-graecum* L. in alloxan diabetic rats for its antioxidative nature and antihyperglycemic potential. They showed increased production of hydrogen peroxide, increased accumulation of malondialdehyde (MDA) and 4-hydroxynonanal (4HNE) and thus immense OS in tissues on one hand, and reduction in SOD), GPx and CAT activities on the other. However, they also showcased the improved levels of H$_2$O$_2$, MDA, and 4HNE and SOD, GPx, and CAT activities as well as transcription of these genes in liver and the brain of diabetic rats treated with *T. graecum* for 15 days.