



***In-vivo* Antioxidant Activity of Ethanol Extract of *Oldenlandia corymbosa* (Linn.) on Streptozotocin with Nicotinamide induced Oxidative Stress in Wistar Rats**

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ABSTRACT

The study was done to investigate the *in-vivo* antioxidant activities of the ethanol extract of the aerial part plant *Oldenlandia corymbosa* in diabetes induced rats with streptozotocin-nicotinamide (STZ with NA). Acute toxic study carried out OECD guideline 420 under this guide line followed the LD₅₀ value fixed 2000mg/kg. This study was conducted for 21 days by treating the animals with plant extract and then induced oxidative stress by administering a single dose of streptozotocin 45mg/kg along with nicotinamide 110mg/kg (p.o). After administration of 100mg/kg and 200 mg/kg (p.o) of ethanol extract of *Oldenlandia corymbosa* (ETOC) to animals, the levels of hepatic and renal GSH, GST, GPx, SOD, CAT and nitric oxide, Thiobarbituric acid reactive substance (TBARS), hydroperoxides (HP), enzymes levels were estimated. The study proves *Oldenlandia corymbosa* (Linn.)'s significant *in-vivo* antioxidant activity helps protect tissues from oxidative stress and decreases a significant amount of hepatic and renal hydroperoxides and thiobarbituric acid substances. A considerable enhance in kidney and liver GSH, SOD, GPx, GST, CAT and NO when compared with diabetic control group was observed in the results. By administering ethanol extract of *Oldenlandia corymbosa* (Linn.) the dose level fixed 100mg, 200 mg/kg, (p.o) and glibenclamide (0.5 mg/kg) the GSH, GST, SOD, GPx, CAT levels considerably enhanced in comparison with diabetic control group. Hence this study concludes that ethanol extract of *Oldenlandia corymbosa* (Linn.) have considerable *in-vivo* antioxidant activity of aids in protecting tissue starting oxidative stress.

Keywords: Ethanol extract; *In-vivo* antioxidant; *Oldenlandia corymbosa*; streptozotocin-nicotinamide (STZ with NA); oxidative stress.

INTRODUCTION

There is prospective evidence that generation of free radicals induce oxidative stress, as a complication association with diabetes (Bisht Shradha *et al.*, 2010). In diabetes, there is an increased production of oxygen free radicals and sharp reduction of antioxidant defenses which leads to oxidative stress (Sanil kumar *et al.*, 2013; Gopala krishnan *et al.*, 2014) Hence, antidiabetic agents with antioxidative properties would be beneficial (Kalva *et al.*, 2016). Oxidative damage is caused due to generation of reactive oxygen species in STZ with NA induced diabetes mellitus (Premanathan *et al.*, 2010; Sharma *et al.*, 2011). Diabetic and experimental animal models exhibits oxidative stress due to persistent of chronic hyperglycaemia, which thereby causes depletion of antioxidative defense system activities and it promotes free radicals generation (Coskun *et al.*, 2004; Abdelmoaty *et al.*, 2010; Adewole *et al.* 2007). Significant antioxidant activity is observed in

many plants extracts and plant products. (Kähkönen *et al.*, 1999; Djeridane *et al.*, 2006). *Oldenlandia corymbosa* (L.) (Rubiaceae) A widely used annual herb, found throughout India. 'Parppatakappullu' plant, a traditional medicine of Kerala clears heat, activates blood circulation, promotes diuresis and thereby reduces stranguria. The plant was proven to preliminary phytochemical screening presents of Alkaloids, Glycosides, Flavonoids, Coumarin and phenol compounds have potent antioxidant activity. This plant fight against appendicitis, pneumonia, hepatitis, cellulites, urinary infection, cholecystitis and snake bite (Joy *et al.*, 1998; Sadasivan *et al.*, 2006; Tanvi Patel *et al.*, 2014). A small annual, perennial herb, thinly to very densely branched near the base; stems erect or level, up to 29.9 cm long, ribbed, glabrous to scabridulous to glandulous-pubescent. Leaves sessile, linear to narrowly elliptic, 0.5–3.4 cm lengthy 0.05–0.7 cm broad, narrowed to the base. Corolla white to bluish to purplish, tube 0.61–1.0 mm long, parts up to 1.2 mm long, ovate to oblong, about as long as the tube (Mohammad Shahidet *et al.*, 2014). In the original systems of medicine like Unani, Ayurveda and Siddha the plant is being used very specifically. Still enough scientific information to support these claims are not available [Zhang *et al.*, 1998]. As a result, it seemed value to estimate high potential antioxidant activity showed in ethanol extract

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of *Oldenlandia corymbosa* on tissue lipid peroxides and enzymatic antioxidant in STZ and nicotinamide induced diabetic rats.

MATERIALS AND METHODS

Collection and identification of *Oldenlandia corymbosa*

The whole plant of *Oldenlandia corymbosa* (Linn.) was collected from Kayathar, Thoothukudi District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using BSMPUS, Government of India, Tirunelveli District, Tamil Nadu. The mid-air parts of *Oldenlandia corymbosa* (Linn.) were plant dried under shadow, segregated, crushed by a mechanical processor and went through a 40 lattice sifter. The powdered materials of the aerial parts were put away in a hermetically sealed holder.

Preparation of extract

The above powdered materials were standardization of Pharmacognostical evaluation like foreign matter, ash value, moisture content and followed by successively extracted with ethanol (40-60°C) by using Soxhlet apparatus, the hot continuous percolation method until completely extracted (until color of solvent in siphon tube turns colorless). Then by using ethanol plant extract in a rotary evaporator and freeze drying in a lyophilizer, the concentrated extract was made into dry powder. (Devi *et al.*, 2009; Tandon *et al.*, 2008; Pandya *et al.*, 2013). The dried powdered material of *O. corymbosa* was subjected to Soxhlet extraction individually using different solvents which increasing order of polarity of the solvent like non polar to polar. The powdered drug completely extracted with following solvents were used in petroleum ether, ethyl acetate and ethanol in order to increase non polar to polar.

Animal

Albino adult male wistar rats, weighing approximately 150 to 200g, were acclimatized for 7 days at temperature (25±2°C) and relative humidity (55±1%) in a 12-hour light/dark cycle in a room under hygienic condition. They were given access to water and fed with standard pellet diet *ad libitum* (Pereira *et al.*, 2004). The experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), KM College of Pharmacy, Uthangudi, Madurai (Approved number: 661/02/c/CPCSEA& 19/7/2002.)

Acute toxic study of ethanol extracts from aerial part of *Oldenlandia corymbosa*

Healthy adult male Wister albino rats were subjected for acute toxicity studies as per OECD-420 guidelines for the following extracts *Oldenlandia corymbosa*. Five animals were used for each step of the study. The animals were weighed and the extracts were adminis-

tered in a single dose. The starting dose levels selected for the two crude extracts, the study with a dose of 5 mg/kg and the dose was increased step by step to 50, 300 and 2000 mg/kg body weight. The animals dosed fixed to based on the mortality at one step will decide the next step. The procedure flow chart described the procedure followed for each of the starting doses.

Induction of diabetes

After initial determination of 16 h fasting blood glucose levels were determined. (Blood drawn through the tail vein puncture) Animals were given single intra peritoneal injection of STZ at the dose of 45 mg/kg BW in 0.1 M citrate buffer, pH 4.5 with NA at a dose of 110 mg/kg freshly dissolved in cold 0.9% saline. Following injection, animals were carefully observed for first 24 h for evidence of allergic response, behavioral variations and convulsions.

STZ is diabetogenic due to its selective damage of insulin-producing beta cells by inducing necrosis. It is postulated that the selective beta-cell toxicity of STZ is due to the glucose moiety in its chemical structure, which enables STZ to enter the cell via the low affinity glucose transporter GLUT2 in the plasma membrane (Elsner, *et al.*, 2000). Cell death happening due to the rapid and lethal depletion of NAD in pancreatic β -islets is caused by the oxidative stress produced by STZ and depends on DNA alkylation and following activation of poly ADP-ribose synthetase. Nicotinamide-NA (niacinamide, vitamin B3) is a water-soluble amide of nicotinic acid, which is chemically name as pyridine-3-carboxamide. Nicotinamide is a form of Vitamin B3 that may preserve and improve β -cell function. The STZ induced rat rapidly increased stress it affect multi organ disorder and further more it promote the death condition to avoid this death additionally administered nicotinamide and control the stress and death condition. After 5 days, fasting blood glucose levels were noted and the animals that developed stable hyperglycemia with fasting blood glucose levels more than 200 mg/dl animals were selected for the study (srinivasan *et al.*, 2012; uddand Rao *et al.*, 2016)

Biochemical Assays

Tissue homogenate were prepared with 0.025 M Tris-Hcl buffer (pH 7.5) from the organs like liver, kidney and pancreas which were removed on the 21st day. After centrifugation at 10,000 rpm for 10 minutes, the clear supernatant was used to measure antioxidant activity.

Statistical analysis

Values are expressed as mean \pm standard deviation for six animals in one groups. The results are estimated by one way analysis of difference (ANOVA) followed by post hoc Dunnett's multiple relationship test. Differences involving means were considered to be statistically considerable at ($p \leq 0.05$) (Festing *et al.*, 2006).

RESULTS

Table 2: This shows the comparison of lipid peroxidation activities in the liver of normal group I and other experimental II, III, IV, V groups of rats follows. The levels of lipid peroxidation activities increased in diabetic groups II, III, IV, V compared to normal control group, whereas diabetic groups treated with the ethanol extract (OC) and glibenclamide reversed the altered values, were considerably decreased. (Table 2).

Table 3: Shown the NO, SOD, CAT, GPx, GST and GSH levels was observed in significantly decreased diabetic control on comparison with normal control rats. Administration of ethanol extract and glibenclamide significantly ($P < 0.05$) increased the levels of NO, SOD, CAT, GPx, GST and GSH when compared with diabetic control rats (Table 3).

Table 4: Shows the comparison of lipid peroxidation activities in the Kidney of normal control and treated groups. The levels of lipid peroxidation activities in considerably greater in diabetic group-II compared to normal group-I, whereas the treated with ethanol extract and glibenclamide groups reversed the values, were considerably decreased. (Table 4).

Table 5: The various activities of NO, SOD, CAT, GPx, GST and GSH in the Kidney of normal control and experimental groups are shown in the above table. These enzyme activities showcased a considerable significant decrease ($P < 0.05$) in the diabetic control group than the normal control group. Administration of ethanol extracts of *Oldenlandia corymbosa* to diabetic rats considerably ($P < 0.05$) better antioxidant activities in the kidney when compared with diabetic group II (Table 5).

Table 6: Shows the comparison of the lipid peroxidation activities in the pancreas of normal control and treated groups of rats. The levels of lipid peroxidation in considerably greater in diabetic group compared to normal control, whereas diabetic rats administered with the ethanol extract (OC) and glibenclamide restored the altered values, were considerably decreased (Table 6).

Table 7: The various activities of NO, SOD, CAT, GPx, GST and GSH in the pancreas of normal group I and treated groups, are shown in the below table. These enzymes activities showed a considerable significant decrease ($P < 0.05$) in the diabetic control group than the normal control group. Administration of ethanol extracts of *Oldenlandia corymbosa* to diabetic rats considerably ($P < 0.05$) improved the antioxidant activities in the pancreas when compared with diabetic group (Table.7).

Values are given as means \pm SEM from six rats in each group. # Diabetic control group compared with control group. Group III, IV and V groups compared with group II. NS: Non-significant. * $P < 0.05$, ** $P < 0.01$ when compared to STZ with NA control group

DISCUSSION

The current investigation was carried out to investigate the therapeutic and beneficial effects of ethanol plant extract of *Oldenlandia corymbosa* on antioxidant status in streptozotocin with nicotinamide induced diabetic rats. The herbal plants medicine quality improved further identified and proved the pharmacological evaluation. This plant, *Oldenlandia corymbosa* is proven to have excellent anti-ulcerant and antidiabetic properties and is used in the hills and mountain places of Southern parts of India as their traditional medicine. The acute toxic study of *O. corymbosa* plant ethanol extract fixed the LD₅₀ value followed by the *in-vivo* antioxidant study. The dose level increased at 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg administered to five animals. The animal no motility up to 300mg and one animal dead in 2000mg/kg and other four animals were found alive so the LD₅₀ value fixed in 2000mg/kg body weight. The dose level 1/10th and 1/20th followed by high dose 200mg/kg and low dose 100mg/kg the plant ethanol extract concluded toxic level and safely handle the animals. The intensified free radical production during streptozotocin with nicotinamide induced experimental diabetes causes elevation in the lipid peroxides level and hydroperoxides level by oxidative degradation of polyunsaturated fatty acids (Baynes *et al.*, 1999). This oxidative degradation damage to proteins and DNA, which consequences in a range of complications mediated to diabetes. Free radicals are cytotoxic unstable and highly reactive compounds. The balance among free radical invention and the endogenous antioxidant defense mechanism (Pandey *et al.*, 2010) influence the intensity of tissue damage by the free radicals. TBARS, a major lipid peroxidation product which is most commonly used biomarker to investigate the oxidative damage on lipid. It can respond with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification (Novelli *et al.*, 2014). Several studies have shown raised lipid peroxidation in clinical and experimental diabetes. (Lee *et al.*, 2006.).

The results showed raised lipid peroxidation in the tissues of diabetic group. The elevation of oxygen free radicals in diabetes could be due to increase in blood glucose levels, which upon autoxidation generate free radicals. Streptozotocin has been shown to produce oxygen free radicals (Ivorra *et al.*, 1989). Lipid peroxide mediated tissue damages have been observed in the development of type I and type II diabetes mellitus (DM) (Feillet-Coudray *et al.*, 1999). Earlier studies have reported that there was an improved lipid peroxidation in liver, kidney and pancreas of diabetic rats. The increased free radical production, increases the oxidative stress and lipid peroxidation thus results in the depletion of antioxidant scavenger systems. The key antioxidants like SOD, CAT, GSH, GPx, GST, GSH plays an important role in scavenging the toxic intermediate of incomplete oxidation. Of these, SOD and CAT are the

Table 1: Experimental design

S.NO	GROUP	TREATED
1	Group I	normal control group received normal saline
2	Group II	Diabetic control (STZ 45 mg with NA 110 mg)
3	Group III	Diabetic rats treated with glibenclamide (0.5 mg/kg b.w.)
4	Group IV	Diabetic rats received by low dose 100 mg/kg b.w ETOC extract respectively
5	Group V	Diabetic rats received by high dose 200 mg/kg b.w ETOC extract respectively

Table 2: Abbreviation and Reference

S.NO	Short form	Abbreviation	Reference
1	TBARS	Thiobarbituric acid reactive substances	(Okhawa <i>et al.</i> ,1979)
2	HP	Hydroperoxides(HP)	(Jiang <i>et al.</i> ,1992)
3	NO	nitric oxide(NO)	(Miranda <i>et al.</i> ,2001)
4	GSH	Reduced glutathione	(Regoli, F. and Principato, G., 1995)
5	SOD	superoxide dismutase	(Misra and Fridovich, 1972)
6	CAT	catalase	(Tukahara <i>et al.</i> , 1960)
7	GPx	glutathione peroxidase	(Rotruck <i>et al.</i> , 1973)
8	GST	glutathione-s-transferase	(Habiget <i>et al.</i> ,1974)

Table 3: Comparison of lipid peroxidation activities in the liver

Groups	Liver TBARS (mM/100g tissue)	Liver HP (mM/100g tissue)
Group I	0.94 ± 0.032	76.49 ± 1.085
Group II	3.32 ± 0.118 ^{###}	133.30 ± 1.363 ^{###}
Group III	1.04 ± 0.040 ^{**}	81.04 ± 0.538 ^{**}
Group IV	2.18 ± 0.073 ^{**}	93.52 ± 0.567 ^{**}
Group V	1.86 ± 0.053 ^{**}	87.38 ± 0.604 ^{**}

Values are given as means ± SEM from six rats in each group. # Diabetic control group compared with control group. Group III, IV and V groups compared with group II. NS: Non-significant. *P<0.05, **P<0.01 when compared to STZ with NA control group

Table 4: Comparison of GSH, SOD, CAT, GPx, NO and GST activities in the liver

Groups	LIVER GSH (mg GSH consumed/min/gm)	LIVER SOD (IU/mg)	LIVER CAT (µM of H ₂ O ₂ consumed/min/mg Protein)	LIVER GPx (µg of GSH/min/mg of protein)	LIVER NO (umol/min/g)	LIVER GST (mg/min/100g)
Group I	13.42±0.53	9.75 ± 0.29	82.32 ± 2.13	11.65±0.50	24.75 ± 1.49	7.14 ± 0.29
Group II	08.10±0.25 ^{###}	4.49 ± 0.16 ^{###}	50.46 ± 1.78 ^{###}	05.16±0.17 ^{###}	11.23±1.44 ^{###}	3.43 ± 0.17 ^{###}
Group III	13.11±0.54 ^{**}	8.98 ± 0.33 ^{**}	76.45 ± 2.21 ^{**}	10.94 ±0.31 ^{**}	21.18 ± 1.48 [*]	6.58 ± 0.27 ^{**}
Group IV	09.85±0.47 ^{NS}	5.19 ± 0.21 ^{NS}	59.52 ± 1.88 [*]	07.43 ± 0.33 ^{**}	14.88± 1.37 ^{NS}	4.85±0.14 ^{**}
Group V	10.54±0.54 ^{**}	6.02 ± 0.26 ^{**}	64.31 ± 2.83 ^{**}	08.76 ± 0.59 ^{**}	18.06 ± 1.34 [*]	5.76 ± 0.11 ^{**}

two major scavenging enzymes that remove toxic free radicals. Along with the changes in lipid peroxidation of the diabetic tissues, the key antioxidants also showed decreased activities. The diabetes mellitus have low activity of SOD its reported to previous studies (Pari, *et al.*, 2005). A reduce in the activity of these antioxidants can escort to an excess accessibility of superoxide anion O₂ – and hydrogen peroxide in biological systems, which in revolve produce hydroxyl radicals, subsequent in opportunity and transmission of lipid peroxidation

(Kumuhekare *et al.*, 1992). The provided data in table 2, 4 and 6; STZ-NA diabetic rats had considerable increase in thiobarbituric acid reactive substances (TBARS) level in liver, pancreas and kidney when compared to normal control. Increased levels of lipid peroxidation in streptozotocin with nicotinamide diabetic rats had reported in previous studies (Bonomini *et al.*, 2008). The plant extract completely removed ethanol using rotary evaporator otherwise the ethanol solvent produced toxic effect. The anti-lipid peroxidative property of

Table 5: Comparison of lipid peroxidation activities in the Kidney

Groups	KIDNEY TBARS (mM/100g tissue)	KIDNEY HP (mM/100g tissue)
Group I	1.54 ± 0.05	63.63 ± 1.17
Group II	4.02 ± 0.13 ^{##}	183.72 ± 1.83 ^{##}
Group III	1.69 ± 0.07 ^{**}	67.54 ± 1.21 ^{**}
Group IV	2.89 ± 0.08 ^{**}	77.06 ± 0.73 ^{**}
Group V	2.13 ± 0.06 ^{**}	73.06 ± 0.86 ^{**}

Values are given as means ± SEM from six rats in each group. # Diabetic control group compared with control group. Group III, IV and V groups compared with group II. NS: Non-significant. . *P<0.05, **P<0.01 when compared to STZ with NA control group

Table 6: Comparison of GSH, SOD, CAT, GPx, NO and GST activities in the Kidney

Groups	KIDNEY GSH (mg GSH consumed/min/gm)	LIVER SOD (IU/mg)	LIVER CAT (μM of H ₂ O ₂ consumed/min/mg Protein)	LIVER GPx (μg of GSH/min/mg of protein)	LIVER NO (umol/min/g)	LIVER GST (mg/min/100g)
Group I	12.61 ± 0.36	14.73 ± 0.45	41.6 ± 0.91	8.47 ± 0.30	14.78 ± 0.17	5.98 ± 0.16
Group II	6.90 ± 0.26 ^{##}	6.84 ± 0.26 ^{##}	20.94 ± 0.67 ^{##}	4.63 ± 0.16 ^{##}	8.04 ± 0.12 ^{##}	3.12 ± 0.08 ^{##}
Group III	12.18 ± 0.46 ^{**}	13.62 ± 0.53 ^{**}	38.69 ± 1.81 ^{**}	7.98 ± 0.23 ^{**}	12.68 ± 0.23 ^{**}	5.84 ± 0.23 ^{**}
Group IV	07.86 ± 0.35 ^{NS}	8.16 ± 0.41 ^{NS}	22.85 ± 1.56 ^{NS}	6.02 ± 0.27 ^{**}	9.90 ± 0.21 ^{**}	4.26 ± 0.21 ^{**}
Group V	09.17 ± 0.49 ^{**}	9.32 ± 0.47 ^{**}	28.73 ± 0.67 ^{**}	6.95 ± 0.14 ^{**}	10.58 ± 0.24 ^{**}	4.52 ± 0.24 ^{**}

Values are given as means ± SEM from six rats in each group. # Diabetic control group compared with control group. Group III, IV and V groups compared with group II. NS: Non-significant. . *P<0.05, **P<0.01 when compared to STZ with NA control group

Table 7: Comparison of lipid peroxidation activities in the Pancreas

Groups	Pancreas TBARS (mM/100g tissue)	Pancreas HP (mM/100g tissue)
Group I	0.71 ± 0.03	12.98 ± 0.46
Group II	1.95 ± 0.07 ^{##}	29.19 ± 0.67 ^{##}
Group III	0.74 ± 0.06 ^{**}	13.58 ± 0.57 ^{**}
Group IV	1.46 ± 0.07 ^{**}	17.13 ± 0.86 ^{**}
Group V	1.04 ± 0.10 ^{**}	14.48 ± 2.44 ^{**}

Values are given as means ± SEM from each group provided six rats. # Diabetic group compared with control group. Group III, IV and V groups compared with group II. NS: Non-significant. . *P<0.05, **P<0.01 when compared to STZ with NA control group

ethanol extract of *Oldenlandia corymbosa* in experimental diabetes is explained after, the *Oldenlandia corymbosa* extracts administered orally in diabetic groups of rats which considerably reverts reverse thiobarbituric acid reactive substances values to near normal group values. The preliminary cytoconstituents of *Oldenlandia corymbosato* are alkaloids, glycosides, flavonoids and phenol compounds. The different cells oxidation/reduction and its regulation based on the concentration of nitric oxide (NO). This process involves either against anti-oxidation or it produce oxidative stress (Valko et al., 2007). Furthermore, Nitric oxide has a responsibility as a moderator of physiological and pathological function in islets of langerhans (Vincent et al., 2004). This mechanism may be the exacting importance for patients with diabetes mellitus. The

oxidative stress results in increased utilization of nitric oxide, leading to reduced levels of nitric oxide in liver, kidney and pancreas of streptozotocin-nicotinamide in diabetic group than normal control group. Based on the results observed from table 3, 5 & 7, streptozotocin with nicotinamide diabetic rats treated with *Oldenlandia corymbosa* ethanol extract, the normal tissue organs had nitric oxide levels. This is due to its rapid reaction with oxygen. NO has greater affinity for O₂ than superoxide dismutase for O₂. In fact, for the scavenging duties, the nitric oxide may compete with superoxide dismutase for oxygen (Das et al., 2006). Furthermore, it was reported by that enhanced the antioxidants activity and bioavailability are expected to reduce superoxide production and its produced hydrobiopterin (BH4) a cofactor profitable to stimulate

Table 8: Comparison of GSH, SOD, CAT, GPx, NO and GST activities in the Pancreas

Groups	Pancreas GSH (mg/100g tissue)	LIVER SOD (IU/mg)	LIVER CAT (μ M of H ₂ O ₂ consumed/min/mg Protein)	LIVER GPx (μ g of GSH/min/mg of protein)	LIVER NO (umol/min/g)	LIVER GST (mg/min/100g)
Group I	10.16 ± 0.88	5.45 ± 0.55	17.01 ± 0.39	6.34 ± 0.62	5.23 ± 0.54	5.58 ± 0.52
Group II	5.21 ± 0.44 ^{###}	2.68 ± 0.26 ^{###}	6.98 ± 0.20 ^{###}	2.58 ± 0.12 ^{###}	2.02 ± 0.28 ^{###}	2.42 ± 0.26 ^{###}
Group III	9.82 ± 0.62 ^{**}	5.19 ± 0.50 ^{**}	15.41 ± 0.49 ^{**}	5.83 ± 0.53 ^{**}	5.30 ± 0.52 ^{**}	5.24 ± 0.50 [*]
Group IV	7.10 ± 0.82 ^{NS}	3.77 ± 0.49 ^{NS}	12.95 ± 0.36 [*]	4.72 ± 0.39 [*]	4.22 ± 0.46 ^{**}	4.10 ± 0.48 ^{NS}
Group V	8.32 ± 0.95 [*]	4.56 ± 0.52 [*]	13.45 ± 0.22 [*]	4.86 ± 0.73 [*]	4.62 ± 0.48 ^{**}	4.50 ± 0.54 [*]

Values are given as means ± SEM from six rats in each group. # Diabetic control group compared with control group. Group III, IV and V groups compared with group II. NS: Non-significant. *P<0.05, **P<0.01 when compared to STZ with NA control group

endothelial cells nitric oxide production activity. (Thor et al., 1988) Consequently, the antioxidant properties of *Oldenlandia corymbosa* (Linn.) ethanol extract contribute to the increased bioavailability of nitric oxide. Reduced glutathione is a major intracellular Non-protein sulphhydryl compound and is expected for the most part of vital intracellular hydrophilic antioxidant (Karbach et al., 2014). Reduced glutathione was act like a co-substrate for GPx. It's a cofactor for several enzymes and as a oxidative stress prevent of many cells due to the high intracellular levels. In several degenerative disease conditions like diabetes decreased reduced glutathione content may predispose. The ethanol extract *Oldenlandia corymbosa* high dose significantly increase the activity when compared with low dose.

CONCLUSION

The present study has exposed that ethanol extract of *Oldenlandia corymbosa* (Linn.) have demonstrated significant increase in antioxidant activity and decreased the oxidative stress in SOD, CAT, GST and GPx. We conclude that ethanol extract of *Oldenlandia corymbosa* (Linn.) have considerable antioxidant activities comparable with that of Glibenclamide (standard) and hence the plant extract can be used effectively for therapeutic management of diabetes.

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