Effect of Ethyl Acetate Extract of *Acanthospermum hispidum* on the Levels of SOD and LPO in the Plasma, Liver and Kidney of Experimental Rats

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Abstract- *Acanthospermum hispidum* is reported to have a wide range of biological activities such as antidiabetic, antibacterial, antifungal, antimitotic, antioxidant, antitumours and is used for treatment of asthma. In the present study, we report the effect of ethanol extracts of *S.colubrina* root on free radical scavenging activity, in STZ induced diabetic rats. The lipid peroxidation, superoxide dismutase were measured in liver , kidney and plasma homogenate, Oral administration of single dose of STZ (60mg/kg), caused significant increases in Lipid Peroxidation (LPO),and Superoxide Dismutase (SOD)) levels were significantly decreased. Further, the administration of ethanol extracts of *Acanthospermum* leaf to STZ induced diabetic rats, at a dose of 150, 300 and 450 mg/kg orally for 14 days, led to a significant decrease in lipid peroxidation, and Consequently, superoxide dismutase levels were significantly increased. Glibenclamide was used as a positive control (10 mg/kg). It was observed that the effect of ethanol extracts of *A.hispidum* on superoxide dismutase, levels, were compared to that of those produced by the positive control. The activities of SOD and were found to be increased in extract treated diabetic rats in selected tissues. The increased level of lipid peroxidation in diabetic rats also was found to be reverted back, to near normal state in extract treated groups. Thus, it may be concluded that the ethanol extract of *A.hispidum*, may produce its hypoglycemic effect through antioxidant defense mechanism

Keywords – *Acanthospermum hispidum*, SOD, LPO, Liver and Kidney

I. INTRODUCTION

Diabetes most prevalent disease in the world, affecting 25% of population and afflicts 150 million people, and is set to rise to 300 million by 2025[1]. In India, it is estimated that presently 19.4 million individuals are affected by diabetes, and the figure is likely to go up to 57.2 million by the year 2025[2]. Hyperglycemia alone does not cause diabetic complications. It is rather, the detrimental effect of glucose toxicity due to chronic hyperglycemia, mediated and complicated through augmented oxidative stress[3]. Hyperglycemia increases the production of reactive oxygen species (ROS) inside the aortic endothelial cells. ROSinduced activation of protein kinase-C isoforms, increased

formation of glucose-derived advanced glycation end products, increased glucose flux through aldose reductase pathways and activation of cytokines are some of the known biochemical mechanisms of hyperglycemiainduced tissue and cell damage[4]. Many traditional medicinal plants that possess substantial quantity of antioxidant components have been found to be useful against diabetes and its related complications[5]. Hence, there is a huge prospect of development of potential hypoglycemic agent coupled with antioxidant activity, from traditional medicinal plants to combat diabetes and its complications. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world, for a range of diabetic presentations. *Acanthospermum hispidum* a thorny creeper with bluish violet flowers, more commonly available in Southern India has been used traditionally in Siddha system of medicines, to treat various diseases. Popularly called 'ththuvali' by the local tribes, villagers and herbalogists in Tamilnadu, this ethnobotanical herb in known to have unique medicinal properties[6]. It has been widely used to treat respiratory disorders, especially bronchial asthma. It has hepatoprotective activity[7] antibacterial activity8, antidiabetic activity[9] and antioxidant properties[10], and can treat several kinds of leprosy. Therefore, the present investigation was undertaken to evaluate, the role of ethanolic extract of *S.colubrina* roots (SCREx), in effective management of diabetes through antioxidant defense mechanism.

II. Materials and Methods

2.1 Plant material

Whole plant of *A.hispidum* were collected, in the month of December 2018 from the S.V University and surrounding areas Chittoor District, A.P, India. A voucher specimen was deposited at our laboratory for future reference.

2.2 Preparation of extract

The plant was dried under shade and powdered to coarse particles. The 2 kg powdered plant material was defatted, with petroleum ether (60-80°C) in a Soxhlet extraction apparatus and further, the same amount plant material extracted with ethanol. The ethanol extract, *A.hispidum* (AHEx) was selected for the present study.

2.3 Animals

Wister albino rats (150-200gm) of either sex were procured from RVS Pharmaceutical College, Coimbatore, Tamilnadu. Before and during the experiment, rats were fed with standard diet (Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days at standard environmental conditions of temperature, relative humidity and dark/ light cycle. (12 h light or 12 h dark cycle, 25 ± 3°C, 35-60 % humidity). The experiments were conducted according to the ethical norms, approved by the Institutional Animal Ethical Committee guidelines of RVS Pharmaceutical College, Coimbatore (Approval No: IAEC1012/ C06/CPSEA- Coreres- 2008-2009).

2.4 Induction of diabetes

The Group 1,2,3 and 4 animals were then anesthetized with stz (60mg/kg), which was dissolved in saline immediately before use, and injected intraperitoneally. After 2 days, rats with moderate diabetes having glycosuria, indicated by Benedict's qualitative test and moderate hyperglycemia (180-200mg/dl) were used for the experiment

2.5 Lipid peroxidation

Lipid peroxidation was estimated by the method[11]. Liver homogenate mixed (1.0 ml) with 100 µl of 8.1% sodium dodecyl sulfate (SDS), and 600µl of 20% acetic acid solution was kept for 2 min at room temperature, then 600µl of 0.8% solution of TBA, was added, heated at 95°C for 60 min in water bath and cooled with ice cold water at 4°C. The mixtures of n-butanol, and pyridine (15:1, v/v) were added, shaken vigorously and centrifuged at 10,000 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm. Lipid peroxidation was expressed as nmoles of MDA/mg of protein.

2.6 Superoxide dismutase

The SOD was estimated by the method[12 ,13], based on the reduction of Nitro Blue Tetrazolium (NBT) to water insoluble blue formazan. Liver homogenate (0.5 ml) was taken, and 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 µm NBT, and 0.2 ml of 0.1 mM EDTA was added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm, followed by 5 min at 25°C. The control was simultaneously run without liver homogenate. Units of SOD activity was expressed as, the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units/mg of protein

2.7 Statistical analysis

All the values of the present study were expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's T-test. Differences between groups were considered significant at $p< 0.05$, $p< 0.01$, $p < 0.001$ levels.

III. RESULTS

Table 1:

Effect of Ethyl acetate extract of *Acanthospermum hispidum* on the levels of SOD and LPO in the Liver of different experimental rats (Mean±S.D)

Groups	Lipid peroxidation	SOD
Group 1	2.89.56 ±0.85 [a]	41.52±1.54 [d]
Group2	6.23±0.64 [c]	14.42 ±1.12 [a]
Group3	4.55 ±0.56 [b]	26.45 ±1.72 [b]
Group4	3.13 ±0.32 [a]	36.53 ±0.98 [c]
F-Value	26.462	299.152
Significance	0.000	0.000

T-test: Values are expressed as mean ± SEM (n=6). ****P*<0.0001 compared with diabetic control (one way ANOVA followed by Duncan post-hoc tests)

Table 2:

Effect of Ethyl acetate extract of *Acanthospermum hispidum* on the levels of SOD and LPO in the plasma of different experimental rats (Mean±S.D)

Groups	Lipid peroxidation	SOD
Group 1	3.78 ±0.66[a]	13.36[d] ±1.51[d]
Group2	10.12 ±0.42[c]	3.42 ±0.31 [a]
Group3	7.12[b] ±0.52 [b]	7.22 ±0.61[b]
Group4	4.52 ±0.44[a]	10.42[c] ±1.26[c]
F-Value	149.156	94.12
Significance	0.000	0.000

T-test: Values are expressed as mean ± SEM (n=6). ****P*<0.0001 compared with diabetic control (one way ANOVA followed by Duncan post-hoc tests)

Table 3:

Effect of Ethyl acetate extract of *Acanthospermum hispidum* on the levels of SOD and LPO in the Kidney of different experimental rats (Mean±S.D)

Groups	Lipid peroxidation	SOD
Group 1	2.62 ± 0.44^{a}	36.55 ± 1.99^{c}
Group2	6.02 ± 0.52^{c}	13.23 ± 1.24^{a}
Group3	4.16 ± 0.32^{b}	24.26 ± 1.32^{b}
Group4	2.92 ± 0.41^{a}	33.16 ± 1.22^{c}
F-Value	32.162	128.346
Significance	0.000	0.000

T-test: Values are expressed as mean ± SEM (n=6). ***P<0.0001 compared with diabetic control (one way ANOVA followed by Duncan post-hoc tests)

IV. Discussion

Lipid peroxidation is nothing but the oxidative destruction of polyunsaturated fatty acids. This leads to the changes of structure, fluidity and functions of membrane, damage of nucleic acids[14]. Lipid peroxidation proceeds in there steps namely initiation, propagation and termination mammalian cells have a special antioxidants defence mechanism to minimize the formation of toxic oxy and peroxy radicals. Several studies showed that there will be an increase in lipid peroxidation and the role of antioxidants to minimize the damage[15] ,[16]. In the first half of our study we have characterized the changes of lipid peroxidation and protection of antioxidants status under the influence of plant extracts.

In living systems, free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, mixed function oxidises activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism underlying a number of human neuralgic and other disorders. For instance in diabetes, increased oxidative stress which co-exist radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes and play a role in the long term complication of diabetes [17,18].

V. Conclusion

The increased level of lipid peroxidation in diabetic rats also was found to be reverted back, to near normal state in extract treated groups. Thus, it may be concluded that the ethanol extract of *A.hispidum* , may produce its hypoglycemic effect through antioxidant defence mechanism.The major findings of this study is that, in stz diabetic Wistar rats, *A. hispidum* plant of ethanolic extracts offer significant protection against oxidative stress.

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