IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
October 2018 Vol.:13, Issue:3
© All rights are reserved by Busineni Jayasimha Goud et al.

Role of *Artemisia absinthium* on Lipid Peroxidation in the Brain Tissues of STZ Lured Diabetic Rats

Busineni Jayasimha Goud[*1], Poornima D [2]

**1 and 2 Department of Studies and Research in Biotechnology*
Tumkur University, Tumakuru,
Karnataka, India-572103

Submission: 20 September 2018
Accepted: 27 September 2018
Published: 30 October 2018

www.ijppr.humanjournals.com

Keywords: *Artemisia absinthium,* Malondialdehyde, thiobarbituric acid-reactive substances, streptozotocin.

ABSTRACT

The relation between elevated glucose levels, high free-radical activity, and the resultant complications in diabetes is significant for understanding the progression of the disease. The intent of this study is to assess the extent of oxidative damage, through recording the levels of malondialdehyde measured as thiobarbituric acid-reactive substances, which is an index of lipid peroxidation, in the brain of diabetic rats that are experimentally subjected to streptozotocin injection. Methanol leaf extract of *Artemisia absinthium* was administered orally (500 mg/kg body weight) and the effect of the extract on plasma glucose, plasma insulin and the levels of thiobarbituric acid reactive substances, was estimated in streptozotocin-induced diabetic rats. Metformin was used as standard reference drug. A significant increase in the activities of plasma insulin and a decrease in the plasma glucose levels was observed in diabetic rats on treatment with 500 mg/kg body weight of Methanol leaf extract of *A. absinthium* and metformin for 8 weeks. Both the treated groups showed a significant decline in the thiobarbituric acid-reactive substances and lipid peroxides formation in the brain, suggesting its role in protection against lipid peroxidation induced membrane damage. Hence, the resultant data propose a possible antiperoxidative effect of Methanol leaf extract of *A. absinthium* in addition to its antidiabetic effect that may be used for therapeutic purposes.

INTRODUCTION

In diabetes mellitus, neurological ramification in the central nervous system is gaining high intentness nowadays. The major intricacies of hyperglycemia include the potentiation of neuronal damage, ischemic events, and stroke as well. In diabetic condition glucose, usage is declined in the brain tissues, leading to increased risk to severe pathological consequences (McCall 1992). Products of lipid peroxidation increased in the brains of Type 1(Kumar and Menon 1993) and Type 2 diabetic mice (Makar *et al.* 1995). Diabetes mellitus is a chronic disorder of metabolism marked by increased glucose levels and associated free-radical production. The free-radical generation produces oxidative stress in all tissues due to glucose autoxidation, activation of polyol pathway, protein glycation, and, the formation of advanced glycated end (AGE) products (Atalay and Laaksonen, 2002). Schwann cells and axonal cells of neurons are sensitive to reactive oxygen species (ROS) associated oxidative free radical damage due to the high content of lipids. Hyperglycemia in diabetes results in peroxidation of lipids in the cell membrane leading to rigidity and impair of cell function.

Lack of relevant counterpoise mechanism against the free-radical production and redox imbalance in the cell leads to the activation of intracellular oxidative stress implicated pathogenesis in diabetes mellitus (Evans *et al,* 2002). The increased production of ROS can damage biomolecules like proteins, lipids, and even DNA. In addition to these changes, oxidative stress-sensitive signaling also alters the gene expression resulting in the alteration of cellular function ultimately damage (Droge 2001). Lipid peroxidation, attributing to free-radical activity, plays a major role in the advancement of complications of diabetes. Although increased levels of lipid peroxidation, because of free radical activity, have been reported in both type 1 and type 2 diabetes with vascular complications (Griesmacher *et al*. 1995; Jennings 1991). Nowadays, the use of alternative medicine and especially the consumption of botanicals have been rising rapidly all around the worldwide, mostly because of the fewer side effects when compared to modern allopathic medicine (Hu *et al*. 2003).

Artemisia absinthium, (Wormwood) is known from ancient times as medicinal and culinary herb has been used for parasite expelling and prescribed as a tonic for a variety of ailments, from headache to dysentery, the use of wormwood extract as a pharmaceutical cure for several different illnesses far back from the Egyptian dynasty of 1600 B.C. Ethnopharmacological evaluation of *A. absinthium* reported, it's *in vitro* free-radical scavenging activity, cognitive enhancement function, neurite outgrowth function,

antiprotozoal activity, antimalarial activity, antifungal activity, antihelmintic activity and it's *in vivo* antimicrobial activity, anti-cancerous activity, hepatoprotective activity, intoxicating effects anti-oxidative stress function, antibacterial activity, antioxidant activity (Goud *et al*., 2015)**.**The experimentation conducted during this time, the preserved brain tissues of different groups were utilized for the estimation of oxidative stress markers in the brain by studying the extent of lipid peroxidation (LPO). Hence, in this study, we have evaluated the levels of Malondialdehyde (MDA) measured as thiobarbituric acid-reactive substances (TBARS) index of lipid peroxidation in STZ induced diabetic rats.

Administration of Methanol extract of *A. absinthium* to STZ induced diabetic rats led to a reduction in blood glucose levels and raised the levels of plasma Insulin. Through this study, we have demonstrated the defective action of Methanol extract of *A. absinthium* on lipid peroxidation in the brain tissues of STZ diabetic rats in comparison with commercial antidiabetic agent Metformin.

MATERIALS AND METHODS

All the chemicals were procured from Sigma Chemical Company (USA) and SISCO, Research laboratory Pvt. Ltd, India).

HUMAN

Methanol leaf extract

Methanol leaf extract of Artemisia absinthium (dry powder) was purchased from Mahaks Herbal & Aromatic Agro Products, Srinagar- Jammu & Kashmir. Herb to product ratio was 8:1, the necessary extract was suspended in 5% Tween-80 in distilled water prior to utilizing.

Experimentation and Lab animals

The 8weeks study of animal experimentation was performed in the Post Graduate Department of Pharmacology Laboratory, Sree Siddaganga College of Pharmacy, Tumkur, with due permission from the Institutional Animal Ethics Committee (IAEC) with registration number: 123/PO/C/99/CPCSEA. Wistar albino rats (Male) 2-3 months of age and weighing about 150-200g were used for the present study. Animals were acclimatized for a week in the animal house, maintained at a temperature of 24-28° C. The light source in the animal room was regulated with 12hour day and night schedule cycle. The animals were fed

with a commercial rodent pellet diet and water ad libitum under strict hygienic conditions by changing the bedding and cleaning the cage with a disinfectant and a detergent regularly.

Grouping of animals

Group 1: Normal rats (N), Group 2: Normal rats treated with 500mg/ kg body weight of MLEAA (NA), Group 3: Diabetic rats (D, Group 4: Diabetic rats treated with 500mg/ kg body weight of MLEAA (DA), Group 5: Diabetic rats treated with 100mg/kg body weight of Metformin (DM).

Induction of diabetes

After one week of acclimatization, the rats were subjected to a 16 h fast. Diabetes was induced in D, DA and DM marked rat groups by a single intraperitoneal injection of freshly prepared STZ with a dosage of 55 mg /kg body weight in 0.05 M citrate buffer pH 4.5 at a volume of 0.1 ml. STZ was first weighed individually in Eppendorf tubes for each animal according to the weight and then solubilized in the buffer, just 15 to 20 minutes prior to injection [11]. Plasma glucose level of each rat was determined after 72 h of STZ administration for confirmation of diabetes. Rats with fasting plasma glucose greater than 300 mg/100 ml were considered diabetic and used for further studies in the present investigation. After confirmation of induction of diabetes, they were sanctioned for a period of 10 days before the commencement of the treatment.

Treatment with MLEAA and Metformin

In the present study the dose of MLEAA (500 mg/kg body weight) used for the treatment in the NA and DA groups is fixed based on the reports of maximum anti-hyperglycemic action shown by MLEAA dose of 500 mg/kg body weight in the earlier investigation conducted on hypoglycemic effects of this plant extract with different doses in STZ induced diabetic rats in 2011 (Goud *et al*., 2011). In the current experimentation, the NA and DA groups were treated daily with MLEAA, orally by gastric intubation with a dose of 500 mg/kg body weight in 5%Tween-80 in distilled water per rat once a day for 60 days. The DM group rats were treated with metformin hydrochloride (100mg/kg body weight) in distilled water once a day for 60 days orally by gastric intubation. Normal (N) and diabetic (D) rats were given distilled water instead of MLEAA.

Brain collection

After the 60 days of experimentation, the animals were starved for 12 h and sacrificed by cervical dislocation and the whole brain was dissected out, washed with ice-cold saline, and used for analysis. 10% homogenate of brain tissue was prepared by homogenization at a 4 °C in 0.15 M KCl. The whole homogenate was used for the estimation of lipid peroxidation (LPO).

Biochemical analysis

Blood glucose was estimated by the GOD-POD enzymatic method (Trinder 1969). Plasma insulin was assayed by the method of Herbert (Herbert *et al.* 1965).

Estimation of lipid peroxidation

Lipid peroxidation in the brain was estimated colorimetrically by measuring the TBARS (Utley *et al.* 1967). To 0.1 ml of 10 % tissue homogenate, 2 ml of 10 % TCA and 4 ml of 0.67 %TBA were added and heated in a water bath for 30 min and cooled. The absorbance of the supernatant was read at 535 nm. The extent of LPO was expressed as nmoles of malondialdehyde (MDA) formed/g tissue, using a molar extinction coefficient of MDA as $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Figure 1 Principle mechanism of the assay of Lipid Peroxidation

Statistical data analysis

The results were expressed as mean ± S.E.M. Research data was analyzed for significant difference using Duncan's Multiple Range (DMR) test ($P < 0.05$) (Duncan 1955).

RESULTS

The level of blood glucose was significantly increased whereas the level of plasma insulin was significantly decreased in diabetic rats [D]. Oral administration of MLEAA and metformin to diabetic rats [D] significantly reversed all these changes to near normal levels.

Lipid Peroxidation

The degree of Lipid Peroxidation (LPO) in brain tissue was studied in five test amasses (groups) and exhibited in representative table and figure. N group rats treated with *A.absinthium* (NA) demonstrated no critical changes in the degree of LPO when contrasted with N rats. STZ actuated diabetic rats (D) (67.42%) demonstrated an eloquent advancement in the degree of LPO in brain tissue contrasted with N rats. *A. absinthium* treatment of in diabetic rats came about a critical decline in LPO in DA rats (35.15 %) when contrasted with D group rats like treatment with metformin in DM group rats. In any case, LPO of DA rat group, it is still notably (8.5 % and 7.34%) more than the N group and DM group rats.

Table 1. Glucose (mg/dl) in STZ lured diabetic rats upon MLEAA treatment

Plasma Glucose in mg /dl		
Group	**Initial day**	**Final day**
N	**82.23±1.21[a]**	**85.80±1.28[a]**
NA	**84.52±0.67[a]**	**83.33±1.54[a]**
D	**325.47±4.16[b]**	**368.84±3.72[b]**
DA	**339.64±4.60[b]**	**90.30±1.34[a]**
DM	**345.17±0.59[c]**	**81.31±1.23[c]**

Test results are conveyed as mean ± S.E.M (n=8). Means with different superscripts inside the segment are fundamentally unique at P<0.05 (Duncan's multiple range test).

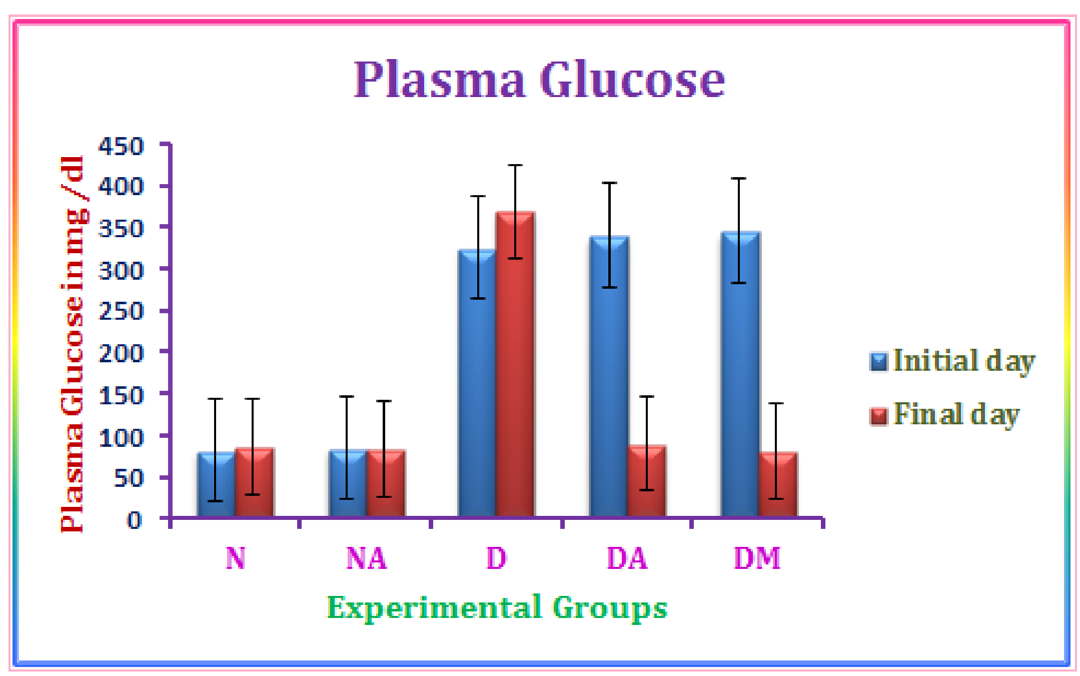

Figure 2 Effect of MLEAA therapy on Glucose levels in STZ abetted diabetic rats.

Table 2 Insulin (µUnits/ml) in STZ lured diabetic rats upon MLEAA treatment

Plasma Insulin (µUnits/ml)		
Group	**Initial day**	**Final day**
N	**37.97±0.46[a]**	**42.27±1.59[a]**
NA	**36.1±0.36[a]**	**41.1±1.05[a]**
D	**14.51±1.12[b]**	**11.07±0.24[b]**
DA	**14.91±0.34[b]**	**34.61±0.88[c]**
DM	**13.31±0.33[C]**	**43.97±0.51[c]**

Test results are conveyed as mean ± S.E.M (n=8). Means with different superscripts inside the segment are fundamentally unique at $P<0.05$ (Duncan's multiple range test).

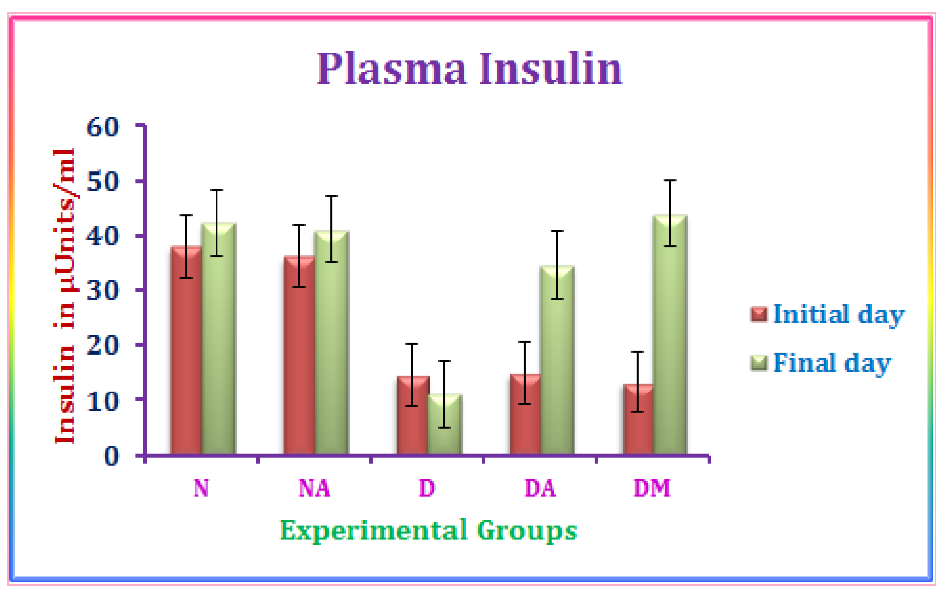

Figure 3 Effect of MLEAA therapy on Glucose levels in STZ abetted diabetic rats.

Table 3 Effect of MLEAA on Lipid peroxidation in STZ induced diabetic rat brain.

Groups	LPO in nmoles of MDA formed /mg protein
N	**0.175±0.012[a]**
NA	**0.168±0.007[a]**
D	**0.293±0.011[b]**
DA	**0.190±0.004[a]**
DM	**0.177±0.013[a]**

Test results are conveyed as mean ± S.E.M (n=8). Means with different superscripts inside the segment are fundamentally unique at $P<0.05$ (Duncan's multiple range test).

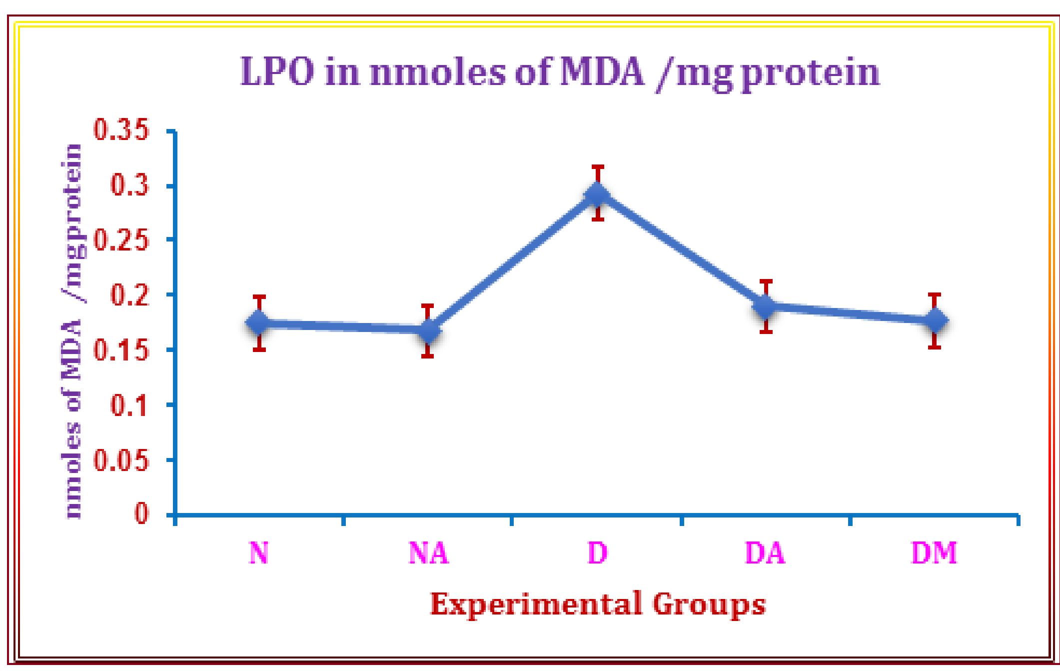

Figure 4 Effect of MLEAA on Lipid peroxidation [LPO] in STZ induced diabetic rat brain.

DISCUSSION

Streptozotocin actuated hyperglycemia in rats is considered to be a good model for the study of diabetes and its complications like insulin deficiency and endogenous oxidative stress etc.

The significant increase in plasma insulin levels of DA group rats like in the DM group rats compared to D group rats may be attributed to the regeneration of the STZ-destructed β-cells which is probably due to the fact that pancreas contains stable cells that have the capacity to regenerate (Cano *et al*., 2008**).** Flavonoids and alkaloids present in the MLEAA may have defended the intact β-cells from further deterioration through oxidative stress. Increased cerebral glucose levels cause oxidation of glucose, production of free radicals, enhancement in lipid peroxidation and glycation proteins, uncontrolled polyol pathway.

Our current reports of expansion in degree of LPO in the brain of D group rats are in concurrence with prior studies (Mushtaq *et al*., 2014, Pari and Murugan, 2007; Evcimen *et al*., 2004) proposing that the expansion in degree of LPO assumes a part in the advancement of diabetic intricacies. The whole plant aqueous extract of wormwood (*A. absinthium*) was reported to reduce LPO in lead-intoxicated animal models (Omar *et al*., 2008). Kostadinović *et al*., 2016, has reported the protective action of *A. absinthium* essential oil (AAEO) on the

antioxidative system of broilers experimentally infected with Eimeria oocysts. Another study by Omar *et al*., 2008 also suggested the protective role of *A. absinthium* (wormwood) extract against lipid peroxidation in lead-induced haematotoxicity. The methanol extract of A. absinthium also been reported to have oxidative stress dissipating action in the brain of rats with cerebral ischemia and reperfusion injury (Boraa and Sharmab, 2010).

The ascertained decline in LPO of DA rat group alike in the DM group when contrasted with the D rat group demonstrates that A. absinthium treatment has given security against free radical interceded injury by its promising antioxidative action and the squashing ability of the free radicals.

CONCLUSION

The study reports that *A. absinthium* provides a significant protection against the issues of hyperglycemia and Lipid peroxidative damage of brain due to oxidative stress in diabetic rats like metformin drug in DM group rats. The above observation shows that the methanol leaf extract of *A. absinthium* contains antiperoxidative activity, which could exert a defensive action against STZ induced pathological alterations in the brain of diabetic rats.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Haritha Busineni and Dr. Nagamma, Director of Sigma Biosciences Research center, for their constant support and inspiration.

REFERENCES

1. Atalay M and Laaksonen DE. 2002. Diabetes- Oxidative stress and physical exercise. *J. Spo. Sci. Med*. 1:1-14.
2. Boraa KS and Sharma A. 2010. Neuroprotective effect of *Artemisia absinthium* L. on focal ischemia and reperfusion-induced cerebral injury. J. Eth. 129(3): 403–409.
3. Cano DA. 2008. Regulated beta-cell regeneration in the adult mouse pancreas. *Dia*. 57: 958-966.
4. Droge W. 2001. Free radicals in the physiological control of cell functions. *Phy. Rev*. 82:47-95.
5. Duncan DB. 1955. Multiple range and multiple tests. *Biometrics*. 42:1–42.
6. Evans JL, Goldfine ID, Maddux BA, and Grodsky GM. 2002. Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes. *Endo. Rev*. 23:599-622.
7. Evcimen DN, Ulusu NN, Karasu C and Doğru B. 2004. Adenosine triphosphatase activity of streptozotocin-induced diabetic rat brain microsomes. Effect of vitamin E. *Gen. Phy. Bio*. 23(3): 347-355.
8. Griesmacher A, Kindhauser M, Andert SE, Schreiner W, Toma C and Knoebl P. 1995. Enhanced serum levels of thiobarbituric-acid-reactive substances in diabetes mellitus. *Am. J.*
9. *Med*; 98:469-75.
10. Goud BJ and Swamy BKC. 2011. Hypoglycemic activity of a Methanol extract of *Artemisia*
11. *absinthium* leaves in experimental rats, *IJAPR*, Vol. 2, Issue.7, 307 – 312.

12. Goud BJ and Swamy BKC, 2015. A review on history, controversy, traditional use, ethnobotany, phytochemistry and pharmacology of *Artemisia absinthium* Linn. *Int. J. Adv. Res. Eng. Appl. Sci.* 4 (5): 77-107.
13. Herbert V, Lan KS, Gottlies CW, and Bleiches SG. 1965. Coated charcoal Immunoassay of Insulin. *J. Cli. End. Met*, 41: 486-492.
14. Hu X, Sato J, Oshida Y, Yu M, Bajotto G, and Sato Y. 2003. Effect of Goshajinki-gan (Chinese herbal medicine): Niu-che-sen-qi-wan) on insulin resistance in STZ induced diabetic rats. *Diab. Res. Clin. Pract*. 59:103-111.
15. Jennings PE, McLaren M, Scott NA, Saniabadi AR, and Belch JJ. 1991. The relationship of Oxidative stress to thrombotic tendency in type 1 diabetic patients with retinopathy. *Diab. Med.* 8:860-865.
16. Kostadinović LJM, Popović SJ, Puvača NM, Čabarkapa IS, Kormanjoš SM, Lević JD. 2016. Influence of Artemisia absinthium essential oil on antioxidative system of broilers experimentally infected with Eimeria oocysts. *Vet. Arh*. 86: 253-264.
17. Kumar JS and Menon VP. 1993. Effect of diabetes on levels of lipid peroxides and glycolipids in rat brain. *Met.* 42:1435-1439.
18. McCall AL, 1992.The impact of diabetes on the CNS. *Diab*. 41:557-570.
19. Makar TK, Hungund BL, Cook GA, Kashfi K, and Cooper AJL. 1995. Lipid metabolism and membrane composition are altered in the brains of Type II diabetic mice. *J. Neu.* 64: 2159-2168.
20. Mushtaq N, Schmatz R, Pereira LB, Ahmad M, Stefanello N, Vieira JM, Abdalla F, Rodrigues MV, Baldissarelli J, Pelinson LP, Dalenogare DP, Reichert KP, Dutra EM, Mulinacci N, Innocenti M, Bellumori M, Morsch VM, Schetinger MR, 2014. Rosmarinic acid prevents lipid peroxidation and increase in acetylcholinesterase activity in brain of streptozotocin-induced di-abetic rats. *Cel. Bio. Fun*. 32(3): 287–293.
21. Omar K, Slimania M, Kroufb D, Seddika L and Aouesa A. 2008. Role of Wormwood (Artemisia absinthium) extract on oxidative stress in ameliorating lead-induced haematotoxicity. *Afr. J. Tra. CAM.* 5(3): 263 – 270.
22. Pari L and Murugan P. 2007. Tetrahydrocurcumin prevents brain lipid peroxidation in streptozotocin-induced diabetic rats. *J. Med. Food*. 10(2): 323-9.
23. Trinder P. 1969. Determination of glucose by glucose oxidase method. *Ann. Clin. Bio*. 6: 24-26.
24. Utley HG, Bernheim F, and P. Hochsein. 1967. Effect of sulphydryl reagents on peroxidation of microsomes. *Arc. Bio.Bio.*118: 29-32.

HUMAN