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EVALUATION OF THE POTENTIAL NEUROPROTECTIVE EFFECT OF *PIMPINELLA TIRUPATIENSIS* ON LIPID PEROXIDATION AND MEMBRANE BOUND ENZYMES ACTIVITY IN RAT BRAIN DURING STZ- INDUCED DIABETES

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ABSTRACT

The role of oxidative stress has been reported in various diabetic complications. The objective of the present study was to investigate the neuroprotective role of *Pimpinella tirupatiensis* aqueous extract on brain lipid peroxidation and membrane bound ATPases in Streptozotocin (STZ) induced diabetic rats. Diabetes was induced in male Wistar rats by a single administration of STZ (40 mg/kg intraperitoneal(i.p). Aqueous extract (750 mg/kg/b.w./day) and Glibenclamide (GLB) (20 mg/kg/b.w./day) were administrated orally by intra oral gastric tube for 30 days. Xanthine oxidase(XOD) activity**,** MDA levels and the membrane bound ATP ases like Na^+/K^+, Mg^{2+} and Ca^{2+} ATP ase, were assayed in the brain tissue. In diabetic rats, we observed that Na^+/K^+, Mg^{2+} and Ca^{2+} ATP ase activities were depleted and XOD activity, MDA levels were up regulated. However with the *Pimpinella tirupatiensis* treatment XOD and MDA levels and Na^+/K^+, Mg^{2+} and Ca^{2+} ATPases activities were came back to normalcy. Our results suggest the ability of *Pimpinella tirupatiensis* extract to modulate XOD ,Na^+ /K^+ , Mg^{2+} and Ca^{2+} - ATP ase activities, and lipid peroxidation in STZ - induced diabetes and thus offers effective management in the treatment of diabetes.

INTRODUCTION

Diabetes is a metabolic disorder that produces various dysfunctions in the body, including vascular dysfunction, retinopathy, nephropathy, peripheral neuropathy, and central nervous system (CNS) dysfunction (Mooradian, 1997; Bhardwaj et al., 1999). Diabetes is also considered to be a risk factor for Alzheimer's disease and other neurodegenerative diseases (Ott et al., 1999; Arvanitakis *et al.*, 2004; Ristow, 2004). Hyperglycemia associated with diabetes increases the glucose autoxidation and protein glycation and the subsequent oxidative degradation of glycated proteins leads to enhanced production of reactive oxygen species (ROS) (Limaye and Sivakami, 2003). The neurological consequences of diabetes mellitus in the central nervous system (CNS) are now receiving greater attention. A variety of structural changes have been described in the CNS of diabetic patients and animals (Tilton et al., 1995) in which glucose utilization could decrease in brain tissue leading to acute potential mechanism for increased vulnerability to acute pathological events during diabetes (McCall,1992). India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes will affect approximately 57 million people by the year 2025 in India.Na^+/K^+-ATPase a membrane linked enzyme that catalyzes the hydrolysis of ATP and couples it to the transport of Na and K across cell membrane there by generating the trans membranous Na+/K+-gradient (Hernandez et. al., 1992). Na^+/K^+-

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ATPase is responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the CNS necessary to maintain neuronal excitability. Na+/K+-ATPase is present at high concentrations in brain, consuming about 40-50% of the ATP generated in this organ (Erecinska and Silver, 1994). Na+/K+-ATPase is implicated in metabolic energy production as well as in the uptake, storage, and metabolism of catecholamine's, serotonin, and glutamate (Carageorgiou et al. 2007). Ca^{2+}-ATPase activity is associated with neuronal excitability, cellular depolarization and fine tuning of Ca^{2+}-channel activity (Lees, 1991). Mg^{2+}-ATPase activity associated with mitochondrial membrane bound enzyme which is involved in turnover of ATP synthesis in conjugation with oxidative phosphorylation.

*Pimpinell*a *tirupatiensis* (Balakrishnan and Subramanyam, 1960) is an herbaceous medicinal plant, distributed on Tirumala hills (1000m above MSL) of chittoor district, Andhra Pradesh (Mahadeva Chetty and Rao, 1990). It is endemic species of Umbellifereae and seasonal occurrence with underground tubers root system (Rangacharyulu *et al.,* 1995). It is used for as a aantifertility anti ulcer and aphrodisiac agent (Vedavathy and Mrudala, 1997). *Pimpinella tirupatiensis* is used to treat cough, stomach, asthma, ulcer. Though there is no scientific evidence to support the antidiabetic property of *Pimpinella tirupatiensis* tribal's of *Tirumala region* continue to use it in the management of diabetes.

MATERIAL METHODS

Procurement of chemicals

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Plant material collection:

Tuberous roots of *Pimpinella tirupatiensis* (Pt) were collected from Tirumala hills, (chittoor district, Andhra Pradesh, India) during the raining season and identified by the taxonomist of the herbarium, department of botany, SV University, Tirupati. A voucher specimen (AECBT-05/2007-2008) was deposited in the department of botany, SV University, Tirupati.

Preparation of plant extract

Pimpinella tirupatiensis tubers were dried at room temperature and tubers were powdered in an electrical grinder then stored at 5^0C until further use. Tubers powder 500g was extracted with distilled water 1L for a period of over 24 hours. After filtration, the residue obtained was given resuspended in equal volume of distilled water for 48 hours and filtered again. The above two filtrates were mixed and the solvent was evaporated in a Rota Vapor (Model No- HS- 2005V) at 50-65^{0}C under reduced pressure and then lyophilized to get a powder and the same was used for the study.

Animals and treatment

Male albino wistar strain rats, aged 3-4 months (200±250 g) were used for the present study. The rats were maintained on standard pellet diet ((M/s Hindustan Lever Ltd., Mumbai) and provided access to water *ad libitum*. They were housed in clean, dry polypropylene cages and maintained in a well ventilated animal house with 12 h light-12 h dark cycle. All the experiments were carried out between 8 am to 10 am in order to avoid circadian rhythm induced changes. The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee(RegdNo.438/01/a/CPCSEA/dt.17.07.2001) in its resolution number 09 (iii)/a/CPSCA/IAEC/07-08/SVU/Zool/KSR-DVNK/dated 26/6/08.

Induction of diabetes

The animals were fasted over night and diabetes was induced via single intra peritoneal injection with a freshly prepared STZ (40 mg/kg b.w) dissolved in ice cold 0.1M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12-15 hr as per the method followed by Rakieten et al., (1963). 8 hr after STZ administration the rats were kept for next 24 hr on given 15% glucose solution to prevent hypoglycemia, as STZ is capable of producing fatal

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hypoglycemia due to destruction of β cells which in turn results in to massive pancreatic insulin release. Diabetes was assessed by determining the fasting blood glucose after 48 hr of injection of STZ. The blood glucose levels in STZ rats were increased to markedly higher levels than normal. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (blood glucose level ≥250 g/dl) were selected. *Pimpinella tirupatiensis* aqueous extract given to the diabetic rats for 30 days.

Experimental design

The rats were divided into 5 groups, six rats in each group and treated as follows:

1. **Group I- Normal control (NC) :** Six rats were received the 0.9%Nacl / kg bodyweight via orogastric tube for a period of one month.
2. **Group II -Diabetic control (DC) :** Six rats were used as diabetic control rats by the injection of STZ (40 mg / kg b.w.) intraperitonially to the fasted rats.
3. **Group III - (**Pt.Aq.e**)** : Normal animals were treated orally with 750 mg/kg b.w/day of *Pimpinella* aqueous extract for 30 days
4. **Group IV - (DC+** Pt.Aq.e**)** : Diabetic animals were treated orally with 750 mg/kg b.w/day of *Pimpinella* aqueous extract for 30 days,
5. **Group V (DC+Glb)** : Diabetic animals were treated with 20 mg/kg/day of glibenclamide for 30 days.

After completion of 30 days treatment, the animals were sacrificed by cervical dislocation and the brain tissues were excised at 4^0C. The tissues were washed with ice-cold saline, immersed in liquid nitrogen and immediately stored at -80^0C for further biochemical analysis.

Analytical procedures

The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product MDA by using the method of Ohkawa et al. (1979). Xanthine oxidase activity was assayed by the dye reduction method of Srikanthan and Krishnamurthy, (1955). The activities of Na+/K+, Mg2+ and Ca2+ ATP ases in the brain estimated by the method of Desaiah and Ho (1979). The enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin (BSA) as a standard. The blood glucose levels were measured by using an Accucheck glucometer (Roche, Germany).

Statistical analysis

The data are expressed as Mean values with their SD. In order to carry out statistical analysis, Ms Excel and SPSS 11.5 Version statistical packages are used. In my study the comparison is with respective groups, hence one way analysis of variance technique is applied to observe the significance between the groups. The post – Hoc test Duncan's multiple range test is also performed to know the significant difference among the groups. Entire statistical analysis is carried out at 0.01 levels.

RESULTS

Effect of *Pimpinella tirupatiensis* aqueous extract on the blood glucose levels and body weight changes

The STZ-induced diabetic rats had shown significant increase of blood glucose levels in comparison to normal control rats, which further increased during the experimental period. Oral administration of *Pimpinella tirupatiensis* aqueous extract significantly decreases the blood glucose levels in comparison to diabetic group. However, glibenclamide treatment also decreased the blood glucose levels in a significant manner when compared to diabetic group. The body weight of diabetic rats was also lower than the control group. However, *Pimpinella tirupatiensis* aqueous extract and glibenclamide treatments significantly improved the body weight and brought down towards near normal level (Table 1).

Effect of *Pimpinella tirupatiensis* aqueous extract on XOD, MDA levels and ATPases in STZ-induced diabetic rats

In the present study MDA levels, XOD activity increased and Na^+/K^+-ATPase Ca^{2+}- ATPase activities were decreased significantly ($P<0.01$) in diabetic control rats when compared to normal animals. After treating with *Pimpinella tirupatiensis* aqueous extract MDA levels, XOD activity

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decreased and Na^+/K^+-ATPase, Ca^{2+}- ATP ase activity was significantly increased. The activities of Na^+/K^+-ATPase and Ca^{2+}-ATPase are recovered to normal levels in diabetic rats *treated with Pimpinella tirupatiensis*.. But there was no significant change Mg^{2+}-ATPase in diabetic rats when compared to normal control rats.

DISCUSSION

In the current study, we observed significant increase in blood glucose levels in diabetic rats (Table 1). This may be due to the deterioration of pancreatic β cells due to oxidative stress (kaneto et al., 2001). The elevation of glucose in STZ-treated rats was due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA (Yamamoto, Uchigata, & Okamoto, 1981). We have registered a decrease in body weight in STZ diabetic rats (Table 2). The characteristic loss of body weight associated with STZ-induced diabetes is due to increased muscle wasting in diabetes (Ravi, Ramachandran, & Subramanian, 2004).

When *Pimpinella tirupatiensis* was administered to diabetic rats, the weights seemed to be increased, as was the ability to reduce hyperglycaemia. However, it could not normalise the body weight completely. This study showed that administration of *Pimpinella tirupatiensis* improved the body weight in diabetic rats, which could be attributed to its antidiabetic and antihyperlipidemic role. The administration of *Pimpinella tirupatiensis* to STZ diabetic rats reduced blood glucose levels, in accordance with earlier reports (Dhanabal et al., 2006). In the present study, the blood glucose data clearly showed that dietary *Pimpinella tirupatiensis* restrained the level of hyperglycaemia resulting from the experimental destruction of beta pancreatic cells induced by STZ. The hypoglycaemic effect of *Pimpinella tirupatiensis* increased gradually and was observed to be maximum at the end of the study period, i.e. 30 days. Our findings are similar to reported previously for ginger (Shanmugham et al., 2011). The decrease in blood glucose levels was due to the antidiabetic compounds of *Pimpinella tirupatiensis*. Our results are supporting its use as folklore medicine for the treatment of diabetes. (Table 1).

In the present study the formation of TBARS, a product of lipid peroxidation reaction, was significantly increased in diabetic brain tissues. Our results were also supported by studies of (Manjulata Kumawat et al., 2013). TBARS and hydroperoxides showed high lipid peroxidation. This may be cause the brain contains relatively high concentration of easily peroxidizable fatty acids (Carney et al., 1991). The elevated lipid peroxidation is responsible for the formation of lipid hydroperoxides in membrane and would result in damage of the membrane structure and inactivation of membrane bound enzymes. The accumulation of lipid peroxides adds hydrophilic moieties into the hydrophobic phase and thereby brings about changes in the membrane permeability and cell functions (Pascoe and Redd, 1989). This increased content of MDA was triggered by *Pimpinella tirupatiensis* tuberous root aqueous extract. Similar reports were found in the brain regions of diabetic rats, the elevated level of MDA was significantly decreased in animals fed with ginger (Shanmgham et al., 2010). Safinaz &. Ibrahim., (2008) have reported MDA levels were decreased in brain after supplementation of hesperidin. The anti-oxidant compounds and other pharmacological compounds of *Pimpinella tirupatiensis* extract may inhibit the production of free radicals, and reduced the products of lipid peroxidation (Fig.1).

The results shown that Xanthine oxidase was increased in diabetic rat brain. This result provides support for the previously reported diabetes-induced brain oxidative stress (Traverso et al., 1997). Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid and generates O_2 •− Hydrogen peroxide formed from O_2 •− could be converted into highly reactive •OH leading to oxidative stress (Singh and Pushpa, 2005). After treatment with glibanclamide and *Pimpinella tirupatiensis* aqueous extract to the diabetic animals the activity of Xanthine oxidase was down regulated (Fig.2). This could be due

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to the decreased degeneration of ATP, down regulation of purine metabolism that leads to the low profile of xanthine, hypoxanthine levels which are necessary forhigh activity of XOD. Similar results have been obtained by the treatment with etimode in the diabetic rat CNS (Ates et al., 2006).

Na^+/K^+-ATPase play an important role in the functional activity of nervous cells. The present study has shown that diabetes decreased Na^+/K^+-ATPase activity in brain. This is in agreement with the earlier published data (Franzon et al., 2005). Hyperglycemia has been shown to generate free radicals from auto-oxidation of glucose, formation of advance glycated end products (AGEs) and increased polyl pathway, with concomitant increase in cellular lipid peroxidation and damage of membrane in diabetes (El-Missiry *et al*., 2004). This increased lipid peroxides formation during diabetes disturbes the anatomical integrity of the membrane, leading to the inhibition of several membrane bound enzymes. Previously it has been reported that the inhibition of mouse cerebral Na^+/K^+-ATPase activity by ultraviolet C generated OH^- and a proxyl ($ROO^{-)}$ radical is mediated via lipid peroxidation induced disruption of membrane integrity (Jamme et al., 1995). The reduction in the activity of Na^+/K^+-ATPase observed in diabetic tissue may be due to the membrane peroxidative damage induced by increased lipid peroxidative status.

Na^+- K^+ ATPase are a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It catalyzes the hydrolysis of ATP and couples it to transport of Na^+ and K^+ across the cell membrane, thereby generating the trans membranous Na^+- K^+ gradient (Erecinsk et. al., 1994). The inhibition of such enzyme provokes an increased uptake of Na + and cytosolic free Ca 2+, releasing of acetylcholine and decreasing the membrane potential of synaptosomes from cerebral cortex (Satoh et al., 1992). Decreased Na^+-K^+ ATPase activity leads to neuron-selective lesion in the brain (Lees et al., 1990) suggesting that inhibition of this enzyme may be used as useful indicator of brain neurodegenerative pathophysiology related to memory and cognitive disorders of diabetic state. In the present study, treatment with *Pimpinella tirupatiensis* aqueous extract significantly increased the Na+/K+, ATPase activity in the brain of induced diabetic rats. Mohammad Rizwan Siddiqui (2005) also reported the Na^+/K^+-ATPase decreased in diabetic rat brain by treatment of *Trigonella* seeds. Treatment of the diabetic animals with *Trigonella, Vandate* and combined therapy of *Trigonella* and *Vanadate* restored the decreased activity of Na^+/K^+-ATPase increased lipid peroxides and altered membrane fluidity after 21 days of treatments. It also has antioxidant properties (Genet *et al.,* 2002). A reduction in the production of free radical and lipid peroxides formation by restoring the antioxidant enzymes can beneficially prevent the decreased of activity Na^+/K^+-ATPase enzyme. Administration of *Pimpinella tirupatiensis* aqueous extract increased the Na^+/K^+-ATPase enzyme activity and may help to control free radical generated. In the current study, Mg^{2+}-ATPase activity was not significant change in diabetic rats when compared to normal control rats. Previous studies have suggested that Mg^{2+}-ATPase activity was not significant change in diabetic rats (Liapi et al., 2009**)**.

Diabetes-related ATPase activity changes in cerebral microvessels may depend on altered blood-brain barrier functions (Mooradian et al., 1994). Moreover the decrease in Ca^{2+}-ATPase activity was related to protein glycosylation and lipid peroxidation. Ca^{2+}-ATPase is sensitive to its phospholipids milieu and to polyunsaturated fatty acids. The content of these lipids may change in diabetes and may cause alterations in enzyme activity (Das et al., 2004). The reversal of Ca^{2+}-ATPase activity in *Pimpinella tirupatiensis* aqueous extract treated and glibenclamide treated diabetic rats towards normal level shows the normal functioning of Ca^{2+}-ATPase. This is in agreement with the earlier published data (Anupama et al., 2012). Administrations of *Erythrina variegata* plant extract prevents

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the inhibition of Ca^{2+}-ATPase activity of diabetic rat brains and consequently would attenuate the resultant neurotoxicity. Treatment of diabetic animals with a mixture of certain antioxidants compounds, such as beta-carotene, vitamine E or its analog trolox C, prevented the development of diabetes-induced defects, such as enhanced lipid peroxidation . Previous studies have suggested that some lippholic and water soluble antioxidants, including buthylated hydroxytoluene and vitamine E, are able to prevent the effects of oxidative stress on Ca^{2+}-ATPase activity (Tappia et al., 2001). Recent studies established that Ca^{2+}-ATPase became subnormal in the hippocampus in hyperglycemic rats and administration of Trogonella has prevented the diabetes–induced decreases in enzyme activity (Kumar, 2012). Administration of *Pimpinella tirupatiensis* aqueous extract increased the Ca^{2+}-ATPase enzyme activity and may help to control free radical generated. To conclude the present findings reveals that one month treatment with selected intensity that was adopted is beneficial in countering the alterations in lipid peroxidation and ATPases in wistar strain rats. The antioxidant defense system which plays a major role in countering the free radicals in diabetic rats were reversed back to normal levels when *Pimpinella tirupatiensis* is given. The changes in markers of oxidative stress which include MDA content and antioxidant enzymes indicating efficient adaptative machinery of oxygen species that was operated in the brain tissue in detoxification of oxygen species that are produced due to diabetes. This study drawn a conclusion stating that *Pimpinella tirupatiensis* treatment to diabetic rats may be beneficial to improve the metabolic efficiency and thereby improve the health status. Thus *Pimpinella tirupatiensis* may be used in the formulation of herbal drugs which can be used in the treatment of diabetes. Since *Pimpinella tirupatiensis* exhibited antioxidant and ant diabetic activity, it might be clinically useful in the control of human diabetes. Thus we conclude that successive studies are mandatory to establish the precise nature of *Pimpinella tirupatiensis* active constituents as well as their mechanism of action.

Table 1 Effects of Pimpinella tirupatiensis and glibenclamide treatments on blood glucose level in streptozotocin-induced diabetic rats.

Groups	Blood glucose (mg/dl)		
	0^{th} day	**15th day**	**30^{th} day**
Group I (NC)	80 ±1.365	83 ± 2.45	89 ± 2.295
Group II (DC)	226 ± 2.126 Ψ	263 ± 3.64	79 ± 2.160 Ψ
Group III (DC+Pt.Aq.e)	265 ± 3.048	221 ± 7.865	271 ± 4.196
Group IV (Pt.Aq.e)	82±1.325	82 ± 1.81	176 ± 5.09
Group V (DC+Gli)	263±1.698	172 ± 5.019 ϒ	89± 2.71

All the values are means ± SD of six individual observations.
Ψ Significant at $p < 0.01$ with respect to normal control.
ϒ Significant at $p < 0.001$ with respect to normal control

Table 2 Effects of Pimpinella tirupatiensis and glibenclamide treatments on blood glucose level in streptozotocin-induced diabetic rats.

Groups	Body weight		
	0^{th} day	15th day	30^{th} day
Group I (NC)	194 ± 8.53	203 ± 3.698	213 ± 14.28
Group II (DC)	189 ± 2.56 Ψ	174 ± 4.13	149 ± 6.83
Group III (DC+Pt.Aq.e)	192± 4.845	189 ± 6.526	201 ± 6.055
Group IV (Pt.Aq.e)	199 ± 4.427	190 ± 9.421	198 ± 3.763 ϒ
Group V (DC+Gli)	192± 3.12	194 ± 5.463 ϒ	204 ± 4.57

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All the values are means ± SD of six individual observations.
Ψ Significant at p < 0.01 with respect to normal control.
ϒ Significant at p < 0.001 with respect to normal control.

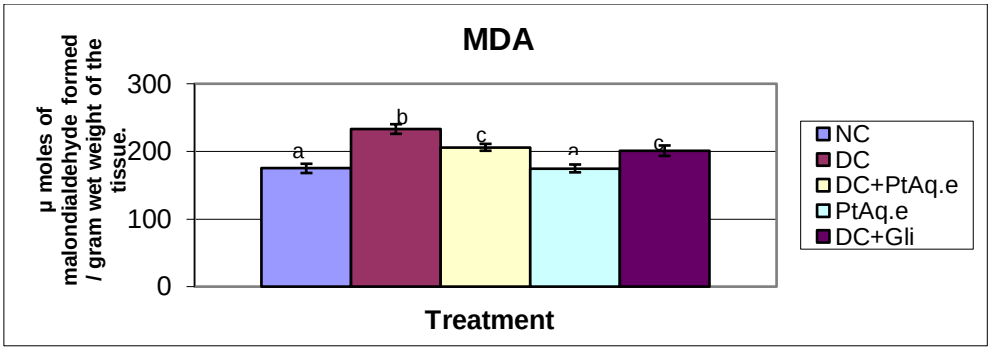

Fig.1: MDA content in the brain Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

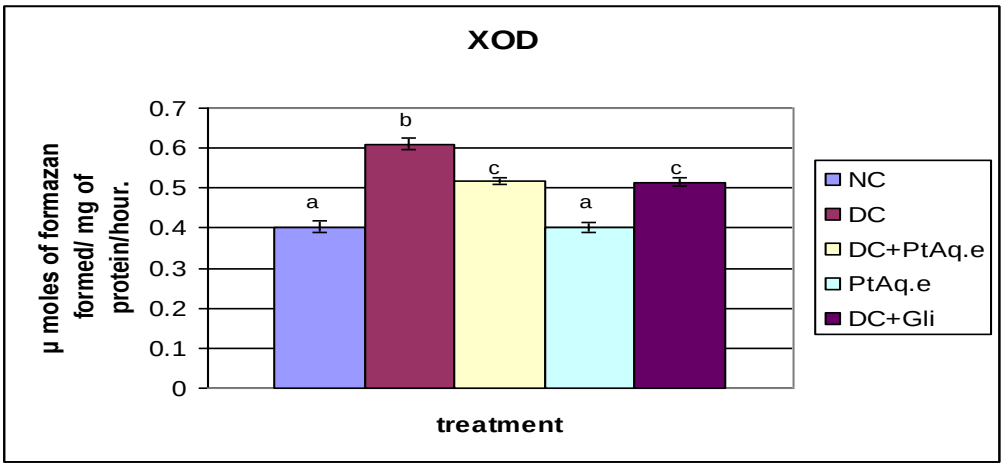

Fig.2: Changes in XOD activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide.* Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

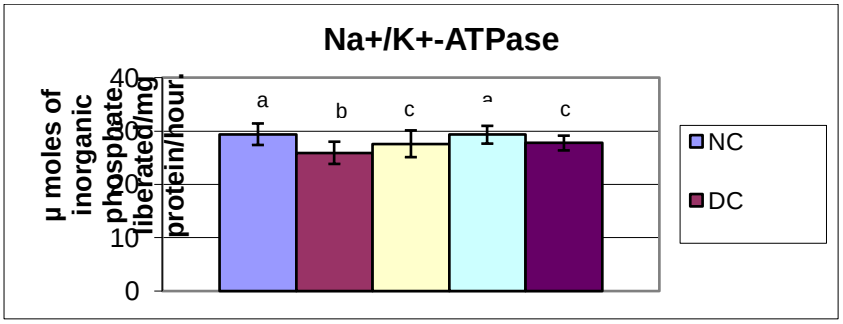

Fig.3: Changes in Na^+/K^+-ATPase activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide* (DC+Gli). Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.01.

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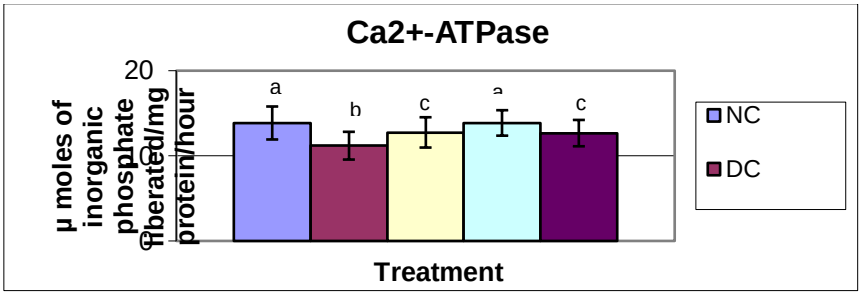

Fig.4: Changes in Ca^{2+} activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide* (DC+Gli). Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.

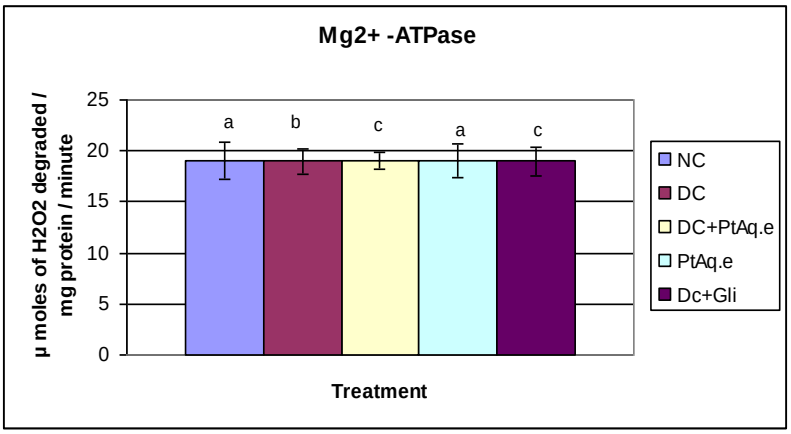

Fig.5: Changes in Mg^{2+}-ATPase activity in the brain of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with *Pt* Aqueous extract (DC+PtAq.e), Control rats treated with *Pt* Aqueous extract (PtAq.e), Diabetic rats treated with *Glibenclamide*(DC+*Gli*). Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at $p<0.01$.

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