Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 967-973

ISSN: 0975-4873

Research Article

Effect of Aqueous Extract of *Passiflora edulis* on Oxidative Stress in Liver and Kidney of Alloxan Induced Diabetic Rats

Devaki Kanakasabapathi[1]*, V.K. Gopalakrishnan[1,2]

[1]*Department of Biochemistry, Karpagam University, Coimbatore-641 021, India.*
[2]*Department of Bioinformatics, Karpagam University, Coimbatore-641 021, India.*

Available Online:

ABSTRACT
Oxidative stress induced by alloxan is implicated in eliciting pathological changes in diabetes mellitus. This study was undertaken to determine the effect of *P. edulis* aqueous leaf extract on oral administration at a concentration of 200 mg/kg body weight for 30 days to alloxan treated animals. Liver and kidney were excised and used for biochemical studies like *invitro* lipid peroxidation, enzymatic antioxidant and non enzymatic antioxidants level.A remarkable increase in the level of enzymatic antioxidants like peroxidase, glutathione peroxidase , superoxide dismutase, catalase , glucose-6-phosphate dehydrogenase, polyphenol oxidase and non enzymatic antioxidant like glutathione, vitamin C and vitamin E were observed. Decrease in basal lipid peroxidation, ascorbate and peroxide induced lipid peroxidation in both the organs were also observed.This study indicates that aqueous leaf extract of *P. edulis* decrease the damage caused by oxidative stress in diabetic rats.

Keywords: Oxidative stress, free radicals, enzymic and non enzymic antioxidants, liver, kidney

INTRODUCTION

In tissues, the oxidation-reduction state also called the redox state depends on the delicate balance between oxidants and antioxidants status at any given time. The oxidants are the free radicals and reactive oxygen or nitrogen species produced in the body as a result of regular intermediary metabolism and other oxidative processes like respiratory bursts seen in neutrophil during phagocytosis. Apart from their roles in the intermediary metabolism and body defense, the oxidants also play a major role as regulatory mediators in signaling processes[1].

Diabetes the chronic metabolic disorder is estimated to affect 4% population worldwide[2]. Oxidative stress is reported to be increased in patients with diabetes mellitus. It is considered as an essential prerequisite for the pathogenesis of this disease[3]. Hyperglycemia can directly cause increased ROS generation. In this condition, glucose can undergo autoxidation and generate •OH radicals. In addition, glucose reacts with proteins in a nonenzymatic manner leading to the development of Amadori products followed by formation of advanced glycation end products (AGEs). In hyperglycemia, there is an enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of $\bullet O_2$-.

Oxygen free radical can initiate peroxidation of lipids which in turn stimulate glycation of proteins, inactivation of antioxidant enzymes and play a role in long term complications of diabetes[4]. Increased oxidative stress as a result of increased free radical formation has also been suggested as a contributor to vascular damage in diabetes[5].

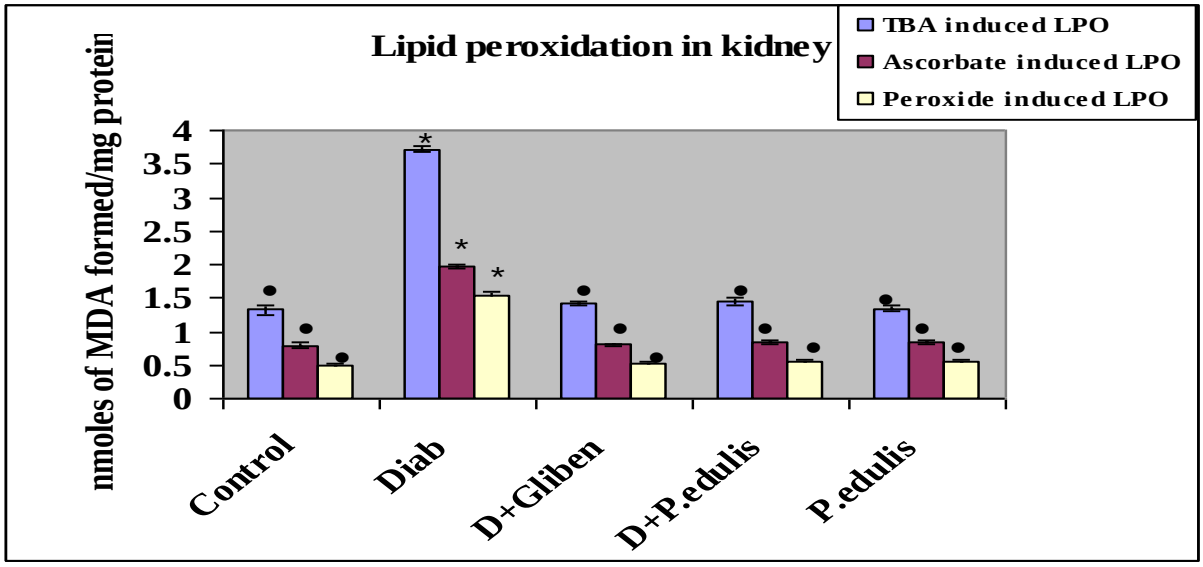
Control of diabetes involves the use of oral hypoglycemic agents and insulin treatment. However natural products derived from plant sources serves the best for the treatment as they are expected to have a similar degree of efficacy without the troublesome side effects associated with modern drug treatment[2]. Eighty percent of the world's population relies primarily on traditional medicines for the health care needs[6]. Historical and traditional plant which is used in folklore medicine is *Passiflora edulis*, which is known as yellow passion fruit. *Passiflora edulis* Sims (Passion fruit) which belongs *Passiflora* genus, comprise about 500 species that are distributed in warm temperatures and tropical regions. It is a vigorous climber. The leaves are evergreen and alternate, 3 lobed leaves when mature. The leaves and stems of *P.edulis* have shown anti-inflammatory, antianxiety, antitumour, antimicrobial and antioxidant activity[5]. The antidiabetic activity of *P.edulis* was well documented[7]. The present study was carried out to find the role of *P.edulis* on antioxidant status and lipid peroxidation metabolism on experimentally induced alloxan diabetic rats.

MATERIALS AND METHODS

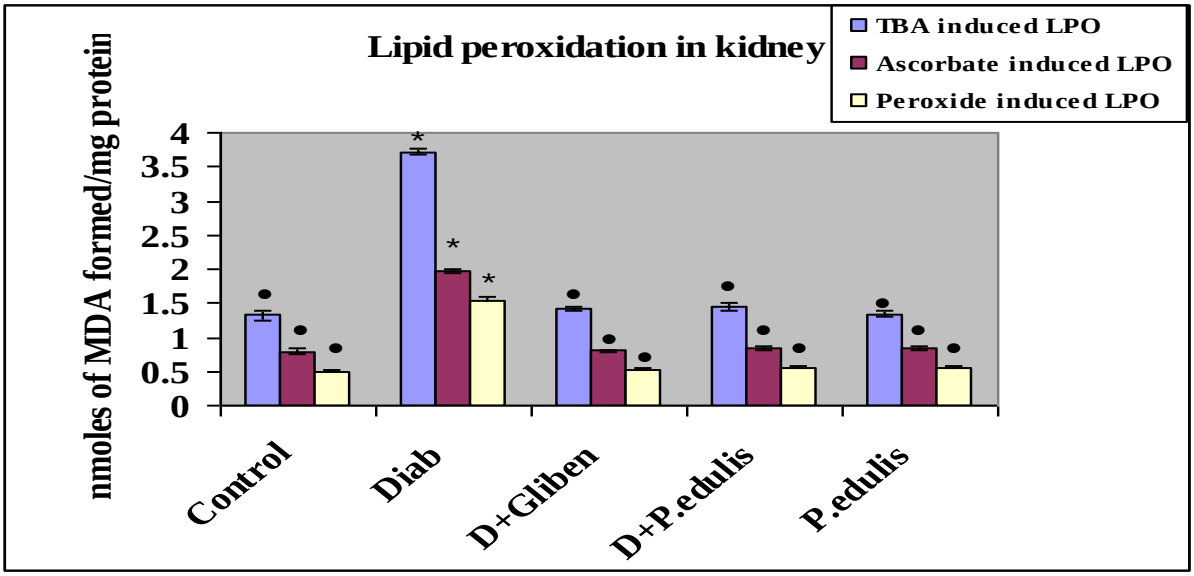
Plant material

Collection of leaves of *Passiflora edulis* was done from a local farm and authenticated by Dr.G.V.S.Moorthy,

*Author for Correspondence



Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common symbol (• , *) differ significantly (DMRT)
Figure 1: Extent of lipid peroxidation in liver of control and experimental rats



Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common symbol (• , *) differ significantly (DMRT)
Figure 2: Extent of lipid peroxidation in kidney of control and experimental rats

Botanical Survey of India, Tamilnadu Agricultural University Campus, and Coimbatore. The voucher number of the specimen is BSI/SRC/73/5/23/9-10/Tech-624.

Extract preparation

The collected leaves were dried at 25^0C for 10 days in the absence of sunlight and powdered well using a mixer. Then they were weighed and kept in an airtight container and stored in refrigerator for future use.

Animals

The adult albino rats of both sexes, weighing about 150-180 g were procured from animal house of Karpagam University, Coimbatore and used for the study. Rats were housed at constant temperature of 22±5°C with a 12-hour light, 12-hour dark cycle. The rats were fed on pellets with free access to tap water. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experimental protocols

A total of 25 rats (15 diabetic surviving rats, 10 normal rats) were used. The rats were divided in to five groups of five animals each. Group I served as control. Group II was diabetic induced. Group III and Group IV were diabetic rats received standard oral hypoglycaemic agent

Table 1: The activities of antioxidant enzymes peroxidase, superoxide dismutase and catalase in liver of control and experimental groups

Groups	Peroxidase[1]	Superoxide diamutase[2]	Catalase[3]
Control	122.56±0.23 [a]	26.38±0.17 [a]	60.78±0.14 [a]
Diabetic control	82.41±0.2 [b]	10.27±0.21 [b]	36.54±0.07 [b]
Diabetic+glibenclamide	119.64±0.19 [c]	24.46±0.26 [c]	59.65±0.23 [c]
Diabetic+*P.edulis*	118.66±0.23 [d]	23.49±0.10 [d]	57.97±0.10 [d]
P.edulis	123.01±0.22 [a]	26.49±0.23 [a]	63.67±0.22 [e]

[1] -Change in absorbance/min/g fresh weight
[2] - Units/g liver tissue
[3] - μ moles of H_2O_2 utilized /min/mg/protein
Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common superscript letter (a-e) differ significantly (DMRT)

Table 2: The activities of antioxidant enzymes peroxidase, superoxide dismutase and catalase in kidney of control and experimental groups

Groups	Peroxidase[1]	Superoxide diamutase[2]	Catalase[3]
Control	130.57±0.08 [a]	31.03±0.03 [a]	56.47±0.04 [a]
Diabetic control	75.88±0.02 [b]	12.43±0.03 [b]	35.37±0.01[b]
Diabetic+glibenclamide	122.01±0.05 [c]	30.52±0.02 [c]	55.09±0.03 [c]
Diabetic+*P.edulis*	121.70±0.05 [d]	28.83±0.02 [d]	53.06±0.05 [d]
P.edulis	130.46±0.09 [a]	30.99±0.04 [a]	55.42±0.02 [c]

[1]-Change in absorbance/min/g fresh weight
[2] - Units/g liver tissue
[3] - μ moles of H_2O_2 utilized /min/mg/protein
Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common superscript letter (a-e) differ significantly (DMRT)

glibenclamide (5mg/kg) and aqueous leaf extract of *P.edulis* (AEPE-200mg/kg) respectively. Group V was normal rats treated with AEPE (200mg/kg) alone. The treatment groups were given the *P. edulis* extracting through oral gastric tube for a period of 30 days.

Biochemical determinations

Liver and kidney were excised immediately after sacrifice, washed with ice cold saline and immediately used for biochemical studies like basal lipidperoxidation[8], ascorbate and peroxide induced lipid peroxidation[9], enzymatic antioxidant like Peroxidase[10], Glutathione peroxidase[11]. Superoxide Dismutase (SOD)[12],Catalase[13], Glucose-6-phosphate dehydrogenase was measured by method[14] and Polyphenol oxidase activity was measured spectrophotometrically[15] and non enzymatic antioxidant like Glutathione[16], Vitamin C[17] and Vitamin E were measured by their standard methods[10].

Statistical analysis

Results are expressed as Mean ± SD of five individual experiment and the statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version (10.0) and the individual comparisons were obtained by the Duncan multiple range test (DMRT)[18]. A value of $p<0.05$ was considered to indicate a significant difference between groups.

RESULTS

Lipid peroxidation was elevated in diabetic rats when compared to the control. *P.edulis* treatment to diabetic rats diminished the extent of lipid peroxidation significantly. The increased levels of TBARS in liver of alloxan induced rats served as an index of elevated lipid peroxidation in the diabetic condition (Figure 1 and 2).

The activities of antioxidant enzymes superoxide dismutase, catalase and peroxidase in liver and kidney are depicted in Table 1 and Table 2. Antioxidant enzymes were decreased in alloxan induced diabetic rats when compared to control group. Glutathione peroxidase, glucose-6-phosphate dehydrogenase and polyphenol oxidase activities were significantly increased in both the organ of *P.edulis* treated diabetic rats (Table 3 and 4).

Non-enzymatic antioxidants like the GSH, Vitamin E and Vitamin C were reduced significantly in liver and kidney ($p<0.05$) of diabetic rats. *P.edulis* treatment improves these antioxidants when compare to diabetic rats. (Table 5 and 6).

DISCUSSION

Hyperglycemia is reported to increase oxidative stress through free radical formation[19]. Endogenous oxygen free radicals scavenging enzymes can respond to such conditions of oxidative stress in diabetes with a compensatory mechanism. Concentration of lipid peroxides was increased in liver, kidney and pancreas of diabetic rats, indicating an increase in free radical generation and / or exhaustion of endogenous antioxidant system[20].

Oxidative stress in liver and kidney

Antioxidant is a substance that protects the biological tissue from damage and can be recycled or regenerated by

Table 3: Activities of antioxidant enzymes glutathione peroxidase, glucose 6 phosphate dehydrogenase and polyphenol oxidase in kidney of control and experimental groups

Groups	Glutathione Peroxidase[1]	Glucose-6-phosphate dehydrogenase[2]	Polyphenol oxidase[3]
Control	50.29±0.15 [a]	94±0.05 [a]	56.52±0.02 [a]
Diabetic control	31.53±0.11 [b]	0.65±0.04 [b]	22.52±0.04 [b]
Diabetic+glibenclamide	47.82±0.15 [c]	0.90±0.04 [a]	53.93±0.05 [c]
Diabetic+*P.edulis*	47.43±0.09 [d]	0.82±0.02 [c]	52.63±0.03 [d]
P.edulis	50.23±0.08 [a]	0.89±0.01 [a]	57.33±0.03 [e]

[1] - µg of GSH/min/mg protein
[2] - mg/g of fresh tissue
[3] - mg/g of fresh tissue
Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common superscript letter (a-e) differ significantly (DMRT)

Table 4: Activities of antioxidant enzymes glutathione peroxidase, glucose 6 phosphate dehydrogenase and polyphenol oxidase in kidney of control and experimental groups

Groups	Glutathione Peroxidase[1]	Glucose-6-phosphate dehydrogenase[2]	Polyphenol oxidase[3]
Control	24.52±0.05 [a]	7.17±0.04 [a]	45.37±0.02 [a]
Diabetic control	18.35±0.03 [b]	5.01±0.02 [b]	22.52±0.04 [b]
Diabetic+glibenclamide	22.99±0.01 [c]	6.25±0.02 [c]	45.65±0.04 [a]
Diabetic+*P.edulis*	22.45±0.04 [d]	6.16±0.02 [d]	45.46±0.05 [a]
P.edulis	24.61±0.02 [a]	7.89±0.04 [c]	46.73±0.04 [c]

[1] - µg of GSH/min/mg protein
[2] - mg/g of fresh tissue
[3] - mg/g of fresh tissue
Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common superscript letter (a-e) differ significantly (DMRT)

Table 5: Levels of Vitamin C, Vitamin E and Glutathione in liver of control and experimental groups

Groups	Vitamin C[1]	Vitamin C[2]	Glutathione[3]
Control	1.94±0.02 [a]	9.27±0.23 [a]	2.98±0.11 [a]
Diabetic control	1.11±0.04 [b]	5.41±0.18 [b]	2.37±0.14 [b]
Diabetic+glibenclamide	1.72±0.05 [c]	8.97±0.19 [ac]	2.87±0.19 [a]
Diabetic+*P.edulis*	1.67±0.05 [cd]	8.77±0.14 [c]	2.83±0.14 [a]
P.edulis	1.95±0.08 [a]	8.99±0.2 [ac]	2.81±0.17 [a]

[1] - mg/g of fresh tissue
[2] - mg/g of fresh tissue
[3] - µg/mg of protein
Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common superscript letter (a-e) differ significantly (DMRT)

Table 6: Levels of Vitamin C, Vitamin E and Glutathione in kidney of control and experimental groups

Groups	Vitamin C[1]	Vitamin E[2]	Glutathione [3]
Control	1.74±0.04 [a]	9.47±0.04 [a]	1.30±0.05 [a]
Diabetic control	1.03±0.01 [b]	6.83±0.31 [b]	0.83±0.07 [b]
Diabetic+glibenclamide	1.65±0.04 [ac]	9.27±0.27 [a]	1.27±0.04 [ac]
Diabetic+*P.edulis*	1.48±0.04 [d]	9.17±0.23 [a]	1.18±0.03[cd]
P.edulis	1.64±0.05 [ac]	9.27±0.14 [a]	1.25±0.04 [ac]

[1] - mg/g of fresh tissue
[2] - mg/g of fresh tissue
[3] - µg/mg of protein
Values are expressed as Mean ± S.D of five individual experiments
Values not sharing a common superscript letter (a-e) differ significantly (DMRT)

biological reducers. The antioxidant system includes numerous enzymes and non enzymic type of antioxidant groups that is located in the cell and in the intracellular fluid. The three most important antioxidant enzymes are SOD, catalase and glutathione peroxidase[21].

Enzymic antioxidant in liver and kidney

The activities of antioxidant enzymes SOD, catalase and peroxidase in liver and kidney are depicted in Table 1 and 2. In physiological condition, SOD is intracellular antioxidants which catalyse the conversion of super oxide anion radical to molecular oxygen and H_2O_2 and thus protects the super oxide anion induced damage[22]. In both liver and kidney a marked decrease in the SOD activity in alloxan induced diabetic rats was observed. The treatment of aqueous extract of *P.edulis* and glibenclamide leads to significant increase in the activity of SOD, suggests a greater level of endogenous antioxidant production resulting in an enhanced free radical scavenging activity. In *P.edulis* alone treated rats significant change in SOD was observed in liver but in kinder.

Catalase is the cellular radical scavenging heme enzyme, which removes hydrogen peroxide and it protects the cell constituents from oxidative damage. Despite the antioxidant enzyme systems, biomolecular damages may still occur and persist within the cell[23] which results in a number of deleterious effects due to the accumulation of hydrogen peroxide[24] that leads to oxidative stress development in diabetic rats[25]. Administration of aqueous extract of *P.edulis* and glibenclamide increased the activity of catalase in diabetic rats. *P.edulis* alone treated rats improved their antioxidant status in liver but not in kidney. This emphasis the protective role of plant extracts on this organ.

A significant decrease in peroxidase activity was observed in alloxan induced diabetic rats[26]. In this study it was reversed by the administration of *P.edulis*. The reversal of peroxidase activity in *P.edulis* treated and glibenclamide treated diabetic rats towards normal level ensures the antioxidant protection against the free radical damage.

Significant decrease in the activities of SOD,catalase, glutathione peroxidase and polyphenol oxidase in liver and kidney tissue of untreated diabetic rats. But the administration of herbal extract which contain phytomedicine and glibenclamide considerably improved the activities of these enzymes[27].

Polyphenols represent a complex group of compounds including several categories such as 4-oxoflavonoids, anthoxyanin and tannins. They act as antioxidants by chelating metals and inhibiting lipooxygenase. They scavenge free radicals[28], block ROS formation and their chain reaction and reduce cellular proliferation. Decreased level of polyphenol oxidase was noted in alloxan induced rats when compared with normal rats. The level of this antioxidant enzyme significantly increased by the administration of aqueous extracts of *P.edulis* and glibenclamide.This shows that the extracts increased the endogenous antioxidant status and hence afford protection against the free radical induced damage. An increase in polyphenol oxidase in normal rat support that the administration of *P.edulis* improves the endogenous antioxidant system.

A decline in the activity of glutathione peroxidase was observed in alloxan induced rats. Our results were also supported by[29,30]. Attainment of normalcy in *P.edulis* extract and glibenclamide treated rats indicate that oxidative stress elicited by alloxan was significantly reduced by this extract. In liver, there was no significant changes seen in plant extract alone treated rats, but a significant increase in their level was found in kidney when it was compared with control indicates the positive role of the extracts in the prevention of oxidant damage.

A decrease in the activity of glucose-6-phosphate dehydrogenase was observed in alloxan induced diabetic rats may also slow down the pentose phosphate pathway in diabetic conditions[31]. Diabetic rats treated with *P.edulis* had a significant increase in glucose-6-phosphate dehydrogenase activity. The mechanism of action elevating glucose-6-phosphate dehydrogenase may be due to increased secretion of insulin, which seems to increase the influx of glucose into the pentose monophosphate shunt in an attempt to reduce high blood glucose levels. This results in an increased production of the reducing agent, NADPH, with concomitant decrease in oxidative stress[26].

Significant decrease in glutathione, glutathione peroxidase, SOD, glutathione reductase and catalase in serum and liver of alloxan induced diabetic rats were noticed and they were restored to their normal level by the administration of 75% methanolic extract of fruits of *Terminalia belerica* was reported[29].

Non enzymic antioxidants in liver and kidney

From the table 5 and 6, it is clear that the alloxan treatment significantly reduced the GSH content ($p<0.05$). The most important mechanism implicated in the diabetogenic action of alloxan is by increased generation of free radical which cause a decrease in plasma GSH concentration[32]. Glibencalmide treated rats showed an increase in GSH level. Similarly plant extracts treated diabetic rats also showed significant increase in GSH. These observations were in accordance with the earlier reports[33,34], indicating depletion in tissue GSH content on alloxan induction in rats, is due to higher levels of free radical generation that convert GSH to its oxidized form. Reduced glutathione detoxifies ROS such as H_2O_2 and lipid peroxides directly in glutathione peroxidase catalyzed reaction.

But treatment of diabetic rats with aqueous extract of *P.edulis*, and glibenclamide, the GSH content was increased significantly when compared with the diabetic groups, indicating that *P.edulis* and glibenclamide could either increase the biosynthesis of GSH or reduce the oxidative stress, which ultimately reduced the degradation of GSH. Glutathione being the most important biological molecule against chemically induced toxicity can participate in the elimination of reactive intermediates by reduction of hydro peroxides in the presence of glutathione peroxidase. Glutathione also function as a free radical scavenger and in the repair of free radical induced biological damage[32].

The levels of Vitamin C in liver and kidney are given in table 5 and 6. The Vitamin C was reduced significantly in alloxan treated rats both in liver and kidney. Upon treatment with the aqueous extract of *P.edulis* and and glibenclamide they were normalized but not to the level

of untreated control. The same was observed in kidney also. The significant reduction in treated groups may be due to the depletion of Vit C during oxidative stress in the removal of oxidative free radicals. Vitamin C serves as an antioxidant by directly scavenging aqueous hydroxyl radical and indirectly by regenerating Vit E and Vit C which defend against the damaging effects of high oxidative stress in diabetes[35].

In comparision with other groups the level of Vit E was reduced significantly in diabetic control groups of both the organs. In humans, the most important lipid soluble exogenous antioxidant is Vitamin E. It is the main antioxidant of lipoprotein and cell membrane. More over Vitamin E has a number of effects at the cellular that are not dependent on its antioxidant activity but by potentially contributing to improve insulin action by inhibiting protein kinase C or by increasing the DAG kinase activity. Protein kinase C impairs insulin action by phosphorylating serine threonine residues in insulin receptor (IR-1) proteins. So increased oxidative stress in diabetes leads to decrease in Vit E content, ultimately it reduces the insulin secretion. But the administration of *P.edulis* increase the insulin secretion there by improves the glucose utilization that reduces the blood glucose level[22]. The result of this study admit the use of *P.edulis* in the amelioration of oxidative stress in the organs like kidney and liver of alloxan diabetic rats and also add an useful information that *P.edulis* have novel principle that have antioxidant activity.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the Chairman and Chancellor, Advisor, Vice Chancellor and Registrar of Karpagam University, Coimbatore, India for providing fund and lab facilities to carry out this work.

REFERENCES

1. Seifried HE, Anderson DE, Fisher EI and Milner JA. A review of the interaction among dietary antioxidants and reactive Oxygen species. J Nutr Biochem 2007; 8: 567-579.
2. Kim SH, Hyun SH and Choung SY. Antidiabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnophramacol 2006; 104: 119-123.
3. Priyanga S, Hemmalakshmi S, Sowmya S, Vidya B, Perumal PC, Gopalakrishnan VK and Devaki K. Quantitative evaluation and *in vitro* free radical scavenging ability of ethanolic stem extract of *Macrotyloma uniflorum* L.Der Pharmacia Lettre 2015; 7: 225-233.
4. Patel SS. Morphology and Pharmacplogy of *Passiflora edulis*. A review. J Herb Med Toxicol 2009; 3: 1-6.
5. Qujeq D and Rezvaniz T. Catalase (antioxidant enzyme) activity in streptozotocin-induced diabetic rats**.** Int J Diabetes & Metabolism 2007; 15: 22-24.
6. Priyanga S, Hemmalakshmi S and Devaki K. Comparative chromatographic fingerprint profiles of ethanolic extract of *Macrotyloma uniflorum* L. leaves and stem. Int J Pharm Cli Res 2014; 6: 288-299.
7. Devaki K, Beulah U, Akila G, Sunitha M and Gopalakrishnan VK. Hypoglycemic activity of *Passiflora edulis* Sims leaf extract in Wistar albino rats. Int Res J Pharm 2011; 2: 170-172.
8. Hogberg J, Larson RE, Kristoferson A and Orrhenices IS. NADPH dependent reductase solublised from microsome by peroxidation and its activity. Biochem Biophys Res Commun 1974; 56: 836-842.
9. Devasagayam TAP and Tarachand. Decreased lipid peroxidation in rat kidney during gestation. Biochem Res Commun 1987; 145: 134-138.
10. Reddy U. Antioxidant vitamins in cardio vascular disease prevention Report on short course on recent advances in vitaminolgy. ANGR Agricultural University, Hyderabad, 1998.
11. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG. Selenium: Biochemical Role as a Component of Glutathione Peroxidase. Science 1973; 179: 588 – 590.
12. Misra HP and Fridovich I. The role superoxide anion in the antioxidation of epinephrine and a simple assay of super oxide dismutase. J Biol chem 1972; 247: 3170-3175.
13. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-394.
14. Balansky D and Berstein RE. The purification and properties of glucose 6 phosphate dehydrogenase from human erythrocytes. Biochem Biophy Acta 1963; 67: 313.
15. Esterbauer H, Schwarzl E and Hayn M. A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid. Anal Biochem 1977; **77:** 486–494.
16. Moron MS, Depierre JW and Mannervik B. Levels of glutathione,glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochem* Biophys Acta 1979; 582: 67-78.
17. Roe JH and Keuther CA. The determination of ascorbic acid in whole blood and urine through 2, 4-dinitrophenylhydrazine derivative dehydroascorbic acid. J Biol Chem 1953; 147: 399-407.
18. Duncan BD. Multiple ranges tests for correlated and heteroscedastic means. Biometrics 1957; 13: 359-364.
19. Ashok P, Kyada A, Subbarao P, Suthar S, Singh D and Vadaliya K. Antioxidant status of polyherbomineral formulation (Gly-13-C) in STZ-diabetic rats. Int J Pharmacol 2010; 6: 157-172.
20. Saha P, Bala A, Kar B, Naskar S, Mazumber UK, Halder PK and Gupta M. Antidiabetic Activity of Cucurbita maxima Aerial Parts. Res J Med plant 2011; 5: 577-586.
21. Marica JB, Veijko B, Jadranka B, Zeiljko M, Ivan J and Zeljko R. Impact of glycemiccontrol on antioxidant enzyme activity in patients with type 2 diabetes mellitus. Diabetologia croatica 2004; 4: 33-34.
22. Shirpoor A, Khadem Ansari MH, Salami S, Ghaderi Pakdel F and Rasmi Y. Effect of Vit E on oxidative

stress in GI tract of diabetic rats. World J Gastroenterol 2007; 13: 4340-4344.
23. Karuna R, Srinivasa Reddy S, Baskar R and Saralakumari. Antioxidant potential of aqueous extract of *Phyllanthus amarus* in rats. Ind J Pharmacol 2009; 4: 64-67.
24. Lee SE, Ju EM and Kim JH. Free radical scavenging and antioxidant enzyme fortifying activities of extracts from Smilax china roots. Exp Mol Med 2001; 34: 263-268.
25. Mallick C, Chatterjee K, Mandal U and Ghosh D. Protective effects of METC, a formulated herbal drug on glycemic indices and testicular dysfunctions in streptozotocin- induced diabetic rat. J Herbs Spices Med Plant 2007. 13: 71-93.
26. Ugochukwu NH, Babady NE, Cobourne M and Gasset SR. The effect of *Gongroneuma Latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetei rats. J Biosci 2003; 28: 1-5.
27. Kaleem M, Asif M, Ahmed Q and Bano B. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Sing Med J 2006; 47: 670-675.
28. Decker EA. Phenolics Prooxidants or antioxidants. Nat Rev 1997; 53: 396-407.
29. Sabu MC and Kuttan R. Antidiabetic activity of *Aegle marmelos* and its relationship with its antioxidant properties. Ind J Physiol Pharmacol 2004; 48: 81-88.
30. Sailaja YR, Baskar R, Saralakumari D. The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. Free Rad Biol Med 2003; 35: 133-139.
31. Abdel-Rahim EA, El-Saadany SS, Abo-Eytta AM and Wasif MM. The effect of sammo administration on some fundamental enzymes of pentose phosphate and energy metabolites of alloxanised rats. Nahrung 1992; 36: 18-24.
32. Dubey SK and Batra AC. Antidiabetic activity of *Thuja occidentalis* Linn. Res J Pharm Tec 2008; 1: 362-365.
33. Kakkar R, Manthe SV, Rathi J, Prasad K and Kalra J. Time course study of oxidative stress in aorta and heart of diabetic rat. Clin Sci 1998; 94: 623-632.
34. Szkudelski T, Kandulska K and Okulicz M. Alloxan in vivo does not only exert deleterious effects on pancreatic B cells. Physiol Res 1998; 47: 343-346.
35. Lai MH. Antioxidant Effects and Insulin Resistance Improvement of Chromium Combined with Vitamin C and E Supplementation for Type 2 Diabetes Mellitus. J Clin Biochem Nutr 2008; 43: 191–198.