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Antioxidant Levels In Dextrose Induced Diabetic Rats Treated With *Passiflora foetida* Plant Extract, Standard Drugs And Silver Nanoparticles

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ABSTRACT

The present study is to investigate the antioxidant activity in dextrose induced diabetic rats. The experimental rats were randomly divided into nine groups. In this groups the rats were treated with *Passiflora fotida* plant extracts, low dose ,medium dose, high doses.Here we treated the diabetic rats with glipizide, sitagliptin, vildagliptin and with silver nanoparticles along with plant extracts. The activity of eglutathione peroxidase, catalase and ascorbic acid was increased by the all drugs. The plant *P.foetida* extract administration increased the antioxidant levels in diabetic rats. The findings of the present investigation demonstrated the antioxidant activity of *Passiflora foetida* in dextrose induced diabetic rats.

KEY WORDS: Diabetes, anti-oxidants, Catalase, Glutathione peroxidise, *Passiflora foetida*.

1. INTRODUCTION

Diabetes mellitus, a leading non communicable disease affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world (Zimmet, 1999). The World Health Organization (WHO) reported that 300 million peoples would suffer from diabetes mellitus by the year 2025 (Pradeepa, 2002). India is one of the leading countries for the number of people with diabetes mellitus and it is estimated that diabetes affects approximately 57 million people by the year 2025 in India (Aravind, 2002). Diabetes mellitus is characterized by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective secretion of insulin. These metabolic disturbances result in acute and long-term diabetic complications.

Reactive oxygen species (ROS) are constantly formed in the human body and are removed by an antioxidant defense system. In healthy individuals the generation of ROS appears to be in approximate balance with antioxidant defense. An imbalance between ROS and antioxidant defenses in favour of the former has been described as oxidative stress. In some human diseases, increased oxidative stress may make an important contribution to disease pathology (Halliwell, 1993, Gutteridge, 1995), ROS are generally cytotoxic because of the oxidative damage they can cause to cellular components. However, at low concentrations, ROS may function as physiological mediators of cellular responses (Bae,1997). Oxidative stress, which is associated with the formation of lipid peroxides, is suggested to contribute to pathological processes in aging and many disease such as diabetes, atherosclerosis and cataract (Jianj, 1992) Increased oxidative stress as a result of increased free radical formation has also been suggested as a contributor to vascular damage in diabetes(Gallou,1993, Jennings,1987, Lyons,1991). Oxidative stress derived from excessive superoxide production and an imbalance in antioxidant enzymes has been related to many other pathologies such as chronic granulomatous diseases, diabetic complications, hepatitis, rheumatoid arthritis, influenza virus, ulcer, pneumonia, HIV infection, cataract and glaucoma (Czene, 1997, Wojtaszek,1997).

Many studies have proposed the mechanism for the role of free radicals in the pathogenesis of various diseases including diabetes mellitus (Paolisso, 1993). However, an array of non-enzymatic antioxidants [vitamin E, vitamin C and reduced glutathione (GSH)] and enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx)] defense mechanism are involved in the protection of free radicals induced oxidative damage. Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins (Maritim, 2003). Recent reports indicate that diabetic complications are associated with overproduction of free radicals and accumulation of lipid peroxidation by-products (Palanduz, 2001). Enhanced oxidative stress has been well documented in both experimental and human diabetes mellitus (Baynes, 1991).

Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes mellitus due to the fact that they are less toxic than oral hypoglycemic agents (Ponnachan,1993; Chattopadhyay,1993).

2. MARERIALS AND METHODS

2.1. Plant material: A sample of fresh leaves of *Passiflora foetida* were collected from the area of botanical gareden, Acharya Nagarjuna University,Guntur. The plant was identified and authenticated by a taxonomist in the university.

2.2. Preparation of plant extract: The fresh leaves were collected and dried under shade and then ground into fine powder using laboratory mortar and pestle. The powder (100g) was macerated in 70% of ethanol and 30% of distilled water at room temperature for 72 h. This was then filtered using a filter paper (Whatmann size no. 1) and the filtrate was evaporated to dryness on water bath at 60ºC to a brown dried residue of 24 g and kept in an air tight bottle until used.

Normal healthy male Wistar albino rats, 9-12 weeks old with an average weight of 200-250gm were procured from the Mahaveer Enterprises, Bagh Amberpet, and Hyderabad. They were housed in polypropylene cages and fed with a standard chow diet and water *ad libitum.* The animals were acclimatized to the conditions by maintaining them at a temperature 25±2˚C and relative humidity 55±10 at 12 hour each at dark and light cycle for about 7 days prior to dosing and during the commencement of experiment. All experimental procedures involving animals were conducted in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) 011/NCP/IAEC/2015..

2.3. Induction of experimental diabetes: dextrose was used to induce diabetes mellitus in normoglycemic male albino wistar rats. A freshly prepared solution of dextrose was given as feed orally at 6.6grams/ rat/ 5ml. After 15 days, rats with moderate diabetes having glycosuria and hyperglycemia were selected for the experiment. A total of 54 rats were used in the present investigation. The animals were randomly divided into 9 groups of 6 rats in each group.

Group I: Normal rats
Group II: Diet induced diabetes diseased rats (diet induced controlled)
Group III: Diet induced diabetes rats treated with plant extract (PSF) (100 mg/kg bw)
Group IV: Diet induced diabetes rats treated with plant extract (PSF) (250 mg/kg bw)
Group V: Diet induced diabetes rats treated with plant extract (PSF) (500 mg/kg bw)
Group VI: Diet induced diabetic rats treated with standard glipizide (4mg/kg bw)
Group VII: Diet induced diabetic rats treated with sitagliptin (0.14mg/100gm bw)
Group VIII: Diet induced diabetic rats treated with vildagliptin (10g/kg bw)
Group IX : Diet induced diabetic rats treated with silver naoparticles(10ml/kg bw)

At the end of the treatment period, all rats were fasted for 12 hours and sacrificed by cervical de capitation. The blood was collected into heparinized tubes and plasma was separated by centrifugation and used for biochemical analysis.

2.4. Estimation of glutathione Peroxidase: The activity of GPx in the erythrocytes and tissues was measured by the method of (Rotruck., 1973).

2.5. Estimation of serum catalase: The activity of catalase in the erythrocytes and tissues was determined by the method of Sinha (Sinha,1972).

2.6. Estimation of Ascorbic acid: Ascorbic acid in the plasma, erythrocytes and tissues was estimated by the method of Roe and Kuether (1943).

3. RESULTS

Table.1. Glutathione Peroxidase, Serum Catalase And Ascorbic Acid Levels In Diabetic Rats

Group	Glutathione Peroxidase(mg/L)	Serum Catalase Levels(MU/L)	Ascorbic Acid (mg/dl)
Group I	92.98±1.65	112.55±1.76	0.50±0.28
Group II	75.00±0.84*	92.10±1.71*	0.24±0.03*
Group III	77.80±1.84*	94.88±1.88*	0.26±0.02*
Group IV	83.48±1.27*	103.72±72.36*	0.34±0.03*
Group V	92.88±1.83	112.43±3.11	0.49±0.05
Group VI	82.02±1.96*	114.21±1.22	0.44±0.02
Group VII	90.98±1.62	108.43±1.42*	0.29±0.01*
Group VIII	88.38±1.35*	104.95±1.67*	0.32±0.02*
Group IX	91.37±1.27	112.62±1.66	0.48±0.02

The mean of glutathione peroxide of normal rats were 92.98mg/dl.The mean of diet induced diabetic rats were 152mg/dl.This mean was significant. Diabetic rats treated with standard drug have mean 124.70 mg/dl which is significant. Diabetic rats treated with plant extract of PSF low, medium and high dose have mean values 127.37mg/dl, 126.42mg/dl and 93.77 mg/dl.Among these mean values high dose mean was non significant. Diabetic rats treated with sitagliptin and vildagliptin have mean values 121.75mg/dl and 124.90mg/dl. Both these values are significant. Diabetic rats treated with silver nanoparticles have mean 135.28 mg/dl. Among these mean values high dose was non-significant and high dose increased the glutathione peroxidise levels and improve the antioxidant activity.

The normal rats serum catalase mean was 112.55MU/L Diet induced rats have mean value 125.MU/L. This value is higher than the normal catalase levels. Diet induced diabetic rats treated with standard drug have mean

121.38MU/L. Diabetic rats treated with plant extracts of PSF have mean values 123.47MU/L, 119.27MU/L and 113.07MU/L. The high dose was non-significant and increased the serum catalase levels.Diabetic rats treated with sitagliptin and vildagliptin have mean values 117.23MU/L and 117.25MU/L. Both were significant mean values. The diabetic rats treated with silver nanoparticles have mean 114.42MU/L. This mean was significant and effective. Among plant extracts high dose was effective and silver nanoparticles also effective when compared to normal mean and improves antioxidant activity.

Above table shows the mean values of Ascorbic acid. Normal rats have mean 0.50 mg/dl. Diet induced diabetic rats have mean 3.50mg/dl. The mean was significant and have variance when compared to the normal mean. Diabetic rats treated with standard drug have mean 2.00mg/dl. This mean also significant. Diabetic rats treated with plant extract of PSF have mean values of 2.89mg/dl, 2.37mg/dl and 0.54mg/dl. Among these high dose was non- significant when compared to normal mean. The diabetic rats treated with sitaglitin and vildagliptin have mean values 1.41mg/dl and 1.50mg/dl. Both these values were significant. Diabetic rats treated with silver nanoparticles have mean 3.30mg/dl. The results showed high dose of *Passiflora foetida* increased the vitamin C levels in diabetic rats.

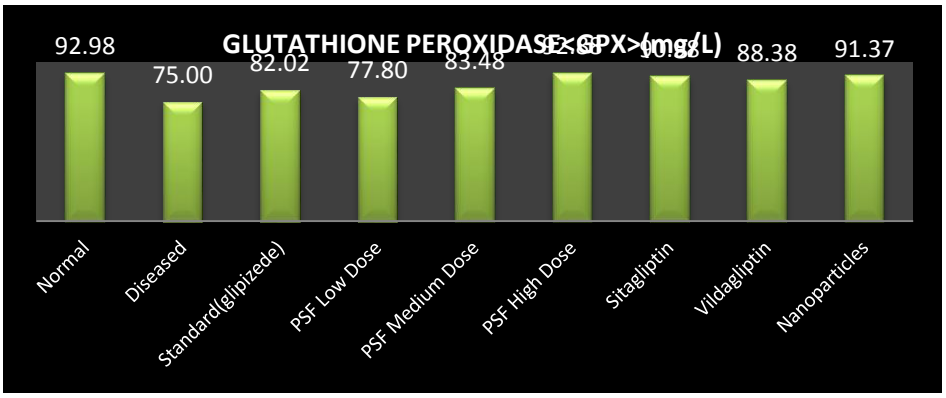
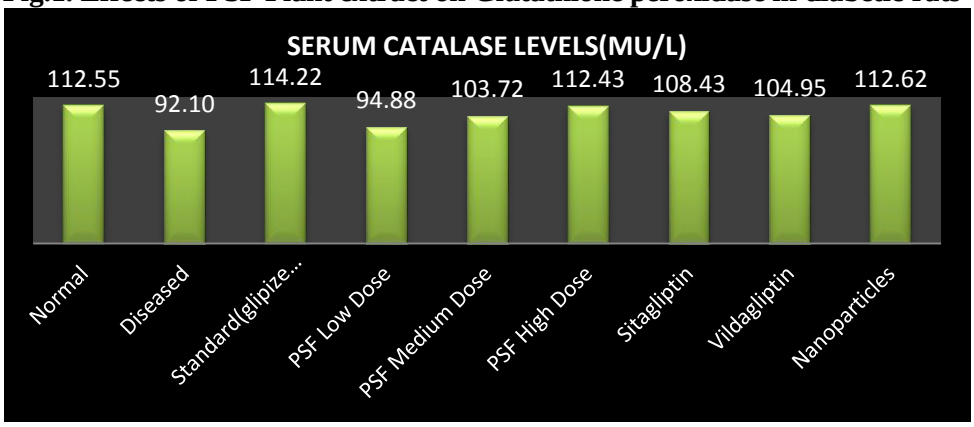
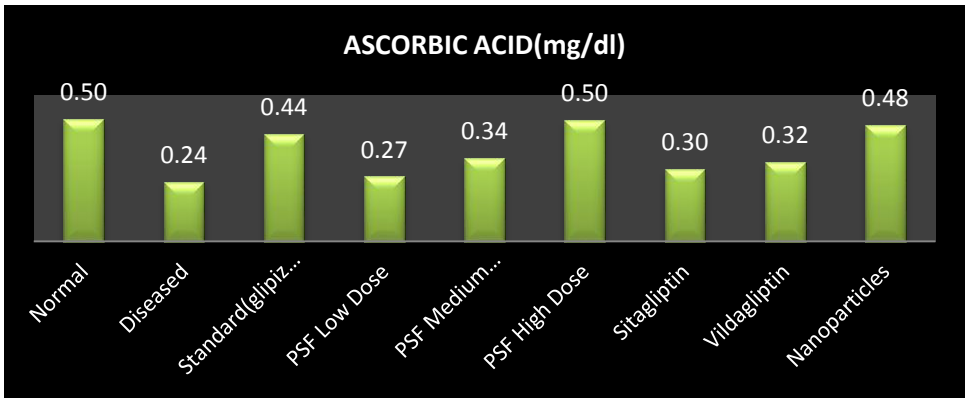


Fig.1. Effects of PSF Plant extract on Glutathione peroxidase in diabetic rats



Effects of PSF Plant extract on Serum Catalase in diabetic



Effects of PSF Plant extract on Ascorbic acid in diabetic rats

Discussion:

GPx is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. In hyperglycemia, glucose undergoes autooxidation and produces superoxide and it produces free radicals that inturn leads to lipid peroxidation in lipoproteins. GPx catalyses the reaction of hydroperoxides with

reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In the present study, the increased the glutathione peroxidise levels with *Passiflora foetida* plant extracts, silver nanoparticles and with standard drugs. This observation perfectly agrees with those of hypoglycemic and antioxidant activity of *Salacia oblonga* (Krishnakumar, 1999).

Vitamin C levels are increased in diabetic rats treated with *passiflra foetida* plant extracts low, medium and high doses. Vitamin C levels are also increased diabetic rats treated with silver nanoparticles and with standard drugs sitagliptin and vildagliptin.Vitamin C levels in plasma significantly decreased in diabetic patients (Wu, 2007; Kashiba, 2002; Peerapatdit, 2006). Decreased vitamin C levels causes hyperlipidemia and hypertension (Welt, 2007; Chen, 2005). Previous studies showed that the fruits and vegetables were important to prevent the diabetes mellitus (Villegas, 2008; Harding, 2008). Ascorbic acid is known to act as an antioxidant both in *in vivo* and *in vitro*. It functions as a free-radical scavenger and successfully prevents detectable oxidative damage under all types of oxidative stress. Ascorbic acid plays an important role in detoxification of reactive intermediates produced by cytochrome P450, which detoxify xenobiotics. Reduction in tissue ascorbic acid was observed in STZ-diabetic rats. The decrease could have been due to increased utilization of ascorbic acid as an antioxidant defense against increased reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of ascorbic acid (Hunt, 1996).

A highly significant reduction in the activity of scavenging mitochondria enzymes is observed in dextrose induced rats. These adverse changes were reversed to near normal values in ethanol extract of *Passiflora foetida* Mitochondria are the energy reservoir of the cell and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death (Sohal, 1994). Sub cellular membrane, associated with thiol bearing enzymes, represents sensitive sites for detoxification causing perpetuation of cellular function (Kyu, 1997). Reactive oxygen species can themselves reduce the activities of antioxidant defence mechanism. In the present study, ethanol extract of *Senna auriculata* have enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

It is well known that serum catalase and GPx play an important role as protective enzymes against free radical formation in tissues (Oberly and Buettner, 1974). Several investigators have reported that the reduced activities of serum catalase genes are induced by free radicals and also by certain humoral factors (Anderson, 1994; Slaga, 1995). The present study indicates the reduction in the activity of serum catalase and glutathione peroxidase in dextrose induced rats. These results reveal the protective role of plant extract in normalizing antioxidant system.

4. CONCLUSION

Therefore, the present investigation imparts novel information on the antioxidant activity of *P.foetida* in dextrose induced diabetic rats Further the oral administration of passiflora extracts, naoparticles and standard drugs to dextrose induced diabetic rats exhibited significant ameliorative potential probably by attenuating the hyperglycemia-mediated oxidative stress.Diabetic rats treated with *passiflora* plant extracts, standard drugs and silver nanoparticles inceased the anti oxidant levels.

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