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Evaluation of *in-vivo* Antioxidant Activity of Methanolic Extract of *Triumfetta rotundifolia (Linn.)* on Streptozotocin induced Oxidative Stress in Wistar Rats.

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Abstract

Obective: The aim of the present study was to investigate the invivo antioxidant activities of the whole plant of methanolic extract of *Triumfetta rotundifolia* in streptozotocin-induced diabetic rats.

Methods: Animals were treated with plant extract for 15 days and then oxidative stress was induced with a single dose of streptozotocin 60mg/kg (p.o). Treated with 200 mg/kg (p.o) of methanolic extract of *Triumfetta rotundifolia* (Linn.) and hepatic and renal thiobarbituric acid reactive substances, hydroperoxides, GSH,SOD,CAT,GP_X,GST,and NO enzymes levels was determined.

Results: The present study revealed that *Triumfetta rotundifolia* (Linn.) has significant *in-vivo* antioxidant activity and can be used to protect tissue from oxidative stress.The result showed a significant decrease in hepatic and renal thiobarbituric acid substances and hydroperoxides. The treatment also resulted in a significant increase in liver and kidney GSH, SOD, CAT, GPx, GST and NO when compared with diabetic control groups.

Conclusion: Methanolic extract of *Triumfetta rotundifolia* (Linn.) in the dose of 200 mg/kg, (p.o) as well as glibenclamid, have improved the GSH, SOD, CAT, GPx, GST levels significantly, which were comparable with diabetic induced rats. Based on this study we conclude that the methanolic extract of *Triumfetta rotundifolia* (Linn.) possesses *in-vivo* antioxidant activity and can be employed in protecting tissue from oxidative stress.

Keywords: *Triumfetta rotundifolia, Streptozotocin, antioxidant enzymes.*

INTRODUCTION

There is increasing evidence that complication related to diabetes are associated with oxidative stress induced by the generation of free radicals (Hussein, 2008)[1]. In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defences (Oberley, 1988)[2]. Hence, compounds with antioxidative properties would be useful antidiabetic agents (Hussein, 2008)[1]. Streptozotocin-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001)[3]. Diabetic and experimental animal models exhibits oxidative stress due to persistent and chronic hyperglycaemia, which there by depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation (Baynes and Thorpe, 1997) [4]. Many plants extracts and plant products have been shown to have significant antioxidant activity (Anjali and Manoj, 1995)[5]. *Triumfetta rotunifolia* (Linn.) belongs to the family Tiliaceae is widely used in Indian traditional medicines and the leaf paste is used to treat rheumatic pain, cough, fever and severe cold (Rajadurai et al. 2009)[6]. The leaf paste is taken along with pepper to treat dyspepsia (Pandikumar et al. 2007)[7]. The bark paste, mixed with hot milk is used internally for treating urinary infections (Silija et al. 2008)[8]. *Triumfetta rotundifolia* Linn. (Liliaceae), is annual slender herb. Its leaves are palmately 5-lobed, scabrous along with smooth beneath, denticulate margin. Its peduncle male flowers contain calyxtube 2-4x3-6 mm, spreading lobes; greenish – yellow corolla, shortly papillose, ovate, acute; lobes. This species is widely distributed throughout India and globally distributed in tropical and subtropical region of Asia, Africa and India. The plant is being used very specifically in the indigenous systems of medicine such as Ayurveda, Siddha and Unani. However, sufficient scientific data to support these claims are still not available. Therefore, it seemed worthwhile to assess anti-oxidant potential of methanolic extract of *Triumfetta ro tundifolia* on tissue lipid peroxidise and enzymatic antioxidant in STZ induced diabetic rats.

MATERIALS AND METHODS

Whole plant of *Triumfetta rotundifolia* (Linn.) were collected from Kalakatu, Tirunelveli District,Tamil Nadu, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India and Palayamkottai. The whole plant leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials (1kg) were successively extracted with methanol (70-80˚C) for 48 hrs by continuous hot percolation method in soxhlet apparatus (Harbone J B, 1984)[9]. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The dried extracts were stored in airtight container.

Animals

Male albino wistar rats each weighing 120-160 gm was obtained from RMMCH in Annamalai University at

Chidambaram (IAEC Approved Number- 767). Rodent laboratory show was access and water *ad libitum* , and rats were maintained on a 12 hour light / dark cycle in a temperature regulated room (20-25^{o}C) during the experimental procedures. The animals were cared for according to the guiding principles in the care & use of animals.

Experimental Design

Rats were divided randomly into four groups of six animals each and treated for two weeks (15 days) as follows. Group I animals served as normal control group received normal saline in a dose of 10ml/kg. Group II served as diabetic Control (STZ-induced diabetic rats, was given physiological saline solution STZ dose of 60mg/kg (p.o). Group III include diabetic rats administered with glibenclamide (10mg/kg b.w./day) in aqueous solution orally for 15 days. Group IV include diabetic rats treated with methnolic extract (200mg/kg b.w./day) suspended in aqueous solution orally for 15 days. After 15 days of the liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

Biochemical Assays:

Liver and kidney tissues nitric oxide(NO) (Miranda et al.,2001)[10], Thiobarbituric acid reactive substances(TBARS) (Okhawa et al.,1979)[11], Hydroperoxides(HP) (Jiang et al.,1992)[12]. Reduced glutathione (GSH) (sedlak and Lindasay, 1968)[13], superoxide dismutase (SOD) (Misra and Fridovich, 1972)[14], catalase (CAT) (Tukahara et al., 1960)[15], glutathione peroxidise (GPx) (Rotruck et al, 1973)[16], glutathione-s-transferase (GST) (Habig et al.,1974)[17].

Statistical analysis:

Values are presented as mean ± standard deviation for groups of six animals. The results were analyzed by one way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test. Differences between means were considered to be statistically significant at ($p \leq 0.05$).

RESULTS:

Table 1 shows the concentration of TBARS and HP in liver and kidney of normal control and experimental groups of rats. The levels of TBARS and HP in diabetic rats were significantly increased than normal control rats, whereas diabetic rats-treated with the methanolic extract and glibenclamide restored the altered values to the near normalcy.

Table 2 shows the concentration of GSH and NO in liver and kidney of control and experimental groups of rats. The decreased of NO and GSH levels was observed in diabetic rats. Administration of *Triumfetta rot undifolia* methanolic extract and glibenclamide tends to bring the NO and GSH levels to near normal.

Table 1. **Effect of thiobarbaturic acid rective substance (TABARS)and hydroperoxides (HP) in liver and kidney of control and experimental groups of rats.**

	Liver		***Kidney***	
Groups	***TBARS (mM/100g tissue)***	***HP (mM/100g tissue)***	***TBARS (mM/100g tissue)***	***HP (mM/100g tissue)***
Normal Control	1.25±0.27	65.83±3.15	2.17±0.07	55.47±2.47
DiabeticControl	2.47±0.21**	94.75±4.27**	3.22±0.16**	72.08±3.59**
Diabetic+GBC	1.35±0.09**	81.97±4.65*	2.37±0.08*	57.35±5.21**
Diabetic+METFRF	0.95±0.067**	63.44±5.24**	2.15±0.09**	40.72±2.84**

Values are given as mean±SD for groups of six animals each. Values are statistically significant at *P<0.005&**P<0.001. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

Table2. Effect of reduced glutathione (GSH) and nitric oxide (NO) in liver and kidney of control and experimental groups of rats.

	Liver		***Kidney***	
Groups	***NO (umol/g)***	***GSH (mg/100g tissue)***	***NO (umol/g)***	***GSH (mg/100g tissue)***
Normal Control	23.86±3.41	67.82±5.28	14.28±0.53	42.96±3.64
DiabeticControl	11.22±3.50**	35.49±3.81**	7.94±0.35**	25.22±2.96**
Diabetic+GBC	21.09±2.77**	50.88±2.95**	12.49±0.33*	33.01±0.97*
Diabetic+METFRF	20.11±2.57**	69.39±4.59**	12.10±0.54*	44.38±4.09**

Values are given as mean±SD for groups of six animals each. Values are statistically significant at *P<0.005&**P<0.001. Diabetic control rats were compared with normal control rats. Experimental groups were compared with diabetic control.

Table3. Effect of superoxide dismutase (SOD) and catalase (CAT) in liver and kidney of control and experimental groups of rats.

	Liver		*Kidney*	
Groups	***SOD (min/mg)***	***CAT (min/mg)***	***SOD (min/mg)***	***CAT (min/mg)***
Normal Control	4.8±0.35	95.14±4.37	8.94±1.28	84.04±5.59
DiabeticControl	3.11±0.09*	62.93±5.08**	5.69±1.07**	64.17±6.28**
Diabetic+GBC	5.72±0.24*	73.32±4.35*	6.08±1.44*	72.13±4.84*
Diabetic+METFRF	6.14±0.24**	101.36±3.87**	9.84±2.11**	94.36±4.61**

Values are given as mean±SD for groups of six animals each. Values statistically significant at *P<0.005&**P<0.001. Diabetic control rats were compared with normal control rats. Experiment groups were compared with diabetic control. Activity expressed as; 50% of inhibition of epinephrine auto oxidation per min for SOD;µmoles of hydrogen peroxide decomposed per min per mg of protein for catalase.

Table4. Effect of glutathione-s-transferase(GST) and glutathione peroxidise (GPx) in liver and kidney of control and experimental groups of rats.

	Liver		*Kidney*	
Groups	***GPx (min/mg)***	***GST (min/mg)***	***GPx (min/mg)***	***GST (min/mg)***
Normal Control	12.08±1.37	10.53±2.11	14.25±2.36	10.37±1.48
DiabeticControl	7.48±1.58**	6.27±1.86**	8.53±1.93**	6.55±2.09**
Diabetic+GBC	8.06±1.97*	7.33±1.68*	10.32±1.38*	7.56±2.11*
Diabetic+METFRF	13.82±2.06**	10.38±1.48**	15.09±2.46**	11.78±2.97**

Values are given as mean±SD for groups of six animals each. Values statistically significant at *P<0.005&**P<0.001. Diabetic control rats were compared with normal control rats. Experiment groups were compared with diabetic control.µmoles of glutathione oxidized per min per mg of protein for GPx;units per min per mg of protein for GST.

Table 3 shows the activities of superoxide dismutase (SOD) and catalase (CAT) in the liver snd kidney of normal control and experimental groups of rats. The activity of SOD and CAT in liver was significantly lower in diabetic control rats compared to control group of rats. After administration of methanolic extract of *Triumfetta rotu ndifolia* as well as glibenclamide in diabetic rats were significant increase the antioxidant enzymes like SOD&CAT.

Table 4 shows the activities of glutathione-s-transferase(GST) and glutathione peroxidise (GPx) in the liver and kidney of normal control and experimental groups of rats. The activity of GST and GPx in liver and kidney was significantly lower in diabetic control rats compared to diabetic induced rats.The GST&GPx levels in liver and kidney were significantly enchanced in methanolic extract of Triumfetta rotundifolia trated rats as well as glibenclamide.

DISCUSSION:

The present study was evaluate the beneficial effects of whole plant of methanolic extract of *Triumfetta rotundifolia* on antioxidant status in STZ-induced diabetic rats. The intensified free radical production during STZ-mediated experimental diabetes resulted in the elevated levels of lipid peroxides and hydroperoxides by oxidative degradation of polyunsaturated fatty acids. These are unstable, cytotoxic and highly reactive, leading to free radical damage to proteins and DNA and finally cause various diabetes-mediated complications. The degree of tissue damage persuaded by free radicals depends on the balance between free radical generation and the endogenous antioxidant defense mechanism (Davi et al., 2005)[18]. One of the most often used biomarker to investigate the oxidative damage on lipid is TBARS a major lipid peroxidation product. It can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification (Pandey and Rizvi, 2010)[19]. According to the provided data in table 1 a notable increase in TBARS level in liver and kidney was observed in STZ-diabetic rats compared with their respective normal controls. Previous study had reported increased levels of lipid peroxidaion in STZ-diabetic rats (Hussein, 2008)[1]. However, the oral administration of *Triumfetta rotundifolia* to the diabetic group of rats significantly reverted back TBARS levels to near normal values which show the anti-lipid peroxidative property of *Triumfetta rotund ifolia* methanolic extract in experimental diabetes.

Nitric oxide is believed to participate in the regulation of the oxidation/reduction potential of various cells and may be involved in either the protection against or the production of oxidative stress within various tissues depending on its concentration (Lamattina et al., 2003)[20]. Furthermore, NO has a dual role as a mediator of physiological and pathological processes in pancreatic islets (Spinas, 1999)[21]. It was generally considered that the persistent hyperglycemia and development of diabetic complication affect no signalling, bioavailability and decrease the most important determinants of NO bioavailability is the reaction of NO with ROS (Mendez and Balderas, 2001)[22]. This mechanism of inactivation of NO may be of particular relevance for

patients with diabetes mellitus (Honing et al., 1998)[23]. The observed decreased levels of NO in liver and kidney of STZ-diabetic group of rats compared the normal control group of rats may represents increased utilization of NO resulting from oxidative stress. In the current results (table 2), STZ-diabetic rats treated with *Triumfetta r otundifolia* methanolic extract had normal plasma and tissue organs NO levels. This effect may be because it rapidly reacting with O_2 The affinity of NO for O_2 is far greater than the affinity of SOD for O_2.In fact, NO may compete with SOD for $O_2^-$and sparing SOD for other scavenging duties (Das and Maulik, 2006)[24]. Furthermore, it was reported by Brown and Hu, 2001, that increases bioavailability of antioxidants are expected to decrease superoxide generation by increasing hydrobiopterin (BH_4) a cofactor needed to stimulate endothelial nitricoxide synthase activity. Therfore, it is likely that the antioxidant effect of *Triumfetta rotundifolia* methanolic extract contribute to the increased bioavailability of NO. GSH is a major intracellular non protein sulphydral compound and is accepted as the most important intracellular hydrophilic antioxidant (Melov2002)[25]. Also, GSH acts as a co-substrate for GPx activity and as a cofactor for many enzymes, stress resistance of many cells is associated with high intracellular levels of GSH. A decreased GSH content may predispose the cells to lower defense against condition of oxidative stress during several degenerative disease conditions including diabetes(Hussein,2008)[1]. In the present study (table 2), the observed elevation in the activities of these antioxidant enzymes in liver and kidney of *Triumfetta rotundifolia* diabetic rats compared to the untreated ones reflects the antioxidant potential of methanolic extract of *Triumfetta rotundifolia*.

Numerous studies have revealed lower antioxidant and enhanced peroxidative status in Type 2 diabetes mellitus (punitha et al., 2005; Pari and Suman,2010)[26].SOD, CAT, GPx and GST are enzymes that destroy the peroxides and play a significant role in providing antioxidant defences to an organism (Punitha et al.,2005)[27]. GPx (Chen and Schopfer, 1999)[28]. CAT (Liedias et al., 1998)[29] are involved in the elimination of H_2O_2 and SOD(McCord et al.,1976)[30] acts to dismutate superoxide radicals to H_2O_2 which is then acted upon by GPx. The function of all three enzymes are interconnected and lowering of their activities result in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats (Kaleem et al.,2006)[31].In the present study table(3&4) the activities of GPx, SOD and CAT in plasma and different tissue organs extracts of the STZ-diabetic rats were significantly lower than their control ones. Impairment of antioxidant machinery may be described by both the damage of antioxidant enzymes caused by protein glycation and consumption by an excess demand (Davi et al.,2005)[16].The compromises in enzymatic antioxidant defense system and alterations in their activities have been implicated in the mechanisms of abnormal tissue function observed in diabetes mellitus(Martin et al.,2003)[32].

CONCLUSION:

In the present study showed that the methanolic extract of *Triumfetta rotundifolia* (Linn.) shows significantly increased in SOD (Super oxide dimustase), catalase, GPx(Glutathione peroxidase and GST(Glutathione-S-transferase). In conclusion of our studies we revealed that methanolic extract of *Triumfetta r otundifolia*(Linn) have significant antioxidant activities as compared with glibenclamide (standard).

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