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ANTIOXIDANT POTENTIAL OF *TRAGIA INVOLUCRATA* LINN ON STREPTOZOTOCIN INDUCED OXIDATIVE STRESS IN RATS

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Tragia involucrata, Streptozotocin- Nicotinamide, Marker enzymes in liver and kidney, Antioxidant activity

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ABSTRACT

In Traditional Ayurvedic system of treatment *Tragia involucrata* Linn. is intended to use for diabetic patients has been screened for antioxidant activity. For antioxidant studies, aqueous ethanolic extract of *Tragia involucrata* (AEETI) was administered orally for 28 days at a dose of 250 and 500 mg/kg body weight to Streptozotocin- Nicotinamide induced Wistar rats. All the animals were sacrificed on the 29th day and the levels of LPO, SOD, CAT, GPx and GSH in kidney and liver of control and experimental rats were studied. The AEETI exhibited significant antioxidant activity showing increased levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and decreased level of lipid peroxidation. These results showed that treatment with AEETI lowers Streptozotocin induced LPO and alters SOD, CAT, GPx and GSH enzymes to reduce oxidative stress.

INTRODUCTION: Reactive oxygen species (ROS) plays major role in the pathogenesis of diabetes and its complications, free radicals have toxic effect on tissues and increase in the blood glucose molecules also in certain condition induces free radical generations. These free radicals by non enzymatic glycosylation of proteins and polyol pathway change the oxidative stress in diabetes. With the help of antioxidants reactive oxygen species can be scavenged. It has been noticed that the alterations in the anti oxidant enzymes activity and its tissue content will results in diabetes. The supplementation with anti oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH), and glutathione peroxidase (GPx) provides greater defence against free radical induced damage [1].

Tragia involucrata Linn (Euphorbiaceae) is a perennial evergreen, climbing hispid herb or shrub with scattered stinging hairs. Widely distributed in the Indian subcontinent, it is locally known as senthoty or poonaikanch chedi in Tamil Nadu [2, 3]. The traditional Ayurvedic practitioners of Kerala use all parts of this herb both externally and internally in the treatment of many common as well as complicated diseases. They also use this herb in treatment of bronchitis, asthma, veneral disease, skin infections and diabetes [4]. The objective of this investigation was to ascertain the scientific basis for the anti oxidant potential of aqueous ethanolic extract of *Tragia involucrata* (AEETI) in Streptozotocin- Nicotinamide induced diabetic rats.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The whole plant of *Tragia involucrata* Linn was collected from the waste lands of Padur and Kelambakkam, Kanchipuram dist., Tamil nadu. The plant material was identified and authenticated by Prof. Dr. D. Narasimhan, Centre for Floristic Research, Madras Christian College, Chennai, Tamil Nadu, India. A

voucher specimen was submitted at C. L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India.

Preparation of Aqueous-Ethanolic extract of *Tragia involucrata* (AEETI): The plant was chopped to small pieces and shed dried. The dried parts were powdered mechanically and weighed quantity of the powder (850 g) was passed through sieve number 40. This powder was packed into soxhlet apparatus and extracted with aqueous-ethanol (80:20) at a temperature of 60°-70°C. The extract was concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator (yield 16.0g) and the semisolid residue was stored in a refrigerator at 2-8°C for use in subsequent experiments.

Experimental animals: Inbred adult Wistar rats (200-250g) of either sex were obtained from the animal house in C. L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bangalore) and drinking water was provided *ad libitum* throughout experimentation period.

Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) Ref No: IAEC/XXX/07/CLBMCP/2010.

Preliminary phytochemical screening [5, 6]: Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, protein, phenols, tannins, flavonoids, terpenes, sterols and saponins in the extract.

Acute oral toxicity studies [7]: The procedure was followed by using OECD guidelines (organization of economic corporation and development) 423 (acute toxic class method).

Three male rats weighing 180-200 gm were used for the study, since the herbal extracts are relatively non toxic, the starting dose level of Aqueous-ethanolic extract of *Tragia involucrata* (AEETI) was selected as 2000mg/kg p.o. and the extract was administered orally to rats which were fasted over night with water *ad libitum*. Body weights of the rats before and after treatment were noted. Any changes in skin and eyes and mucous membrane and also respiratory, circulatory, autonomic, CNS, motor activity, behavior pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted for 14 days.

Induction of diabetes mellitus in experimental animals [8, 9, 10]: Diabetes was induced in overnight fasted healthy inbred Wistar Albino rats (200-250g) of either sex by a single intraperitoneal (i.p) injection of Streptozotocin (STZ) 45 mg/kg, 15 min after the single intraperitoneal (i.p.) injection of Nicotinamide (NA) 110 mg/kg Streptozotocin (STZ) was dissolved in 0.1 M cold citrate buffer, pH 4.5 and Nicotinamide (NA) was dissolved in normal physiological saline.

After injections the animals were free access to food and water. After 4 hours the animals were given with 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycaemia. The development of diabetes is confirmed after 72 hours of the streptozotocin injection and on 7^{th} day after the injections. The animals with fasting blood glucose level more than 200 mg/dl are select for the experimentation.

Experimental design [11, 12]: The animals were divided into 5 groups. Group I consisted of normoglycaemic rats. The remaining 4 groups consisted of 6 STZ-NA induced diabetic rats. The test samples were administered orally by using a gastric lavage.

Group I - Normal rats (Normal control) received 0.5 % SCMC 5 ml/kg p.o.

Group II - STZ-NA induced diabetic rats (Diabetic control) received 0.5 % SCMC 5 ml/kg p.o.

Group III -STZ-NA induced diabetic rats received AEETI 250 mg/kg p.o.

Group IV - STZ-NA induced diabetic rats received AEETI 500 mg/kg p.o.

Group V- STZ-NA induced diabetic rats received Glibenclamide 0.5 mg/kg p.o.

The above mentioned treatment schedule was followed for the respective group of animals for 28 days.

Preparation of liver homogenate [13, 14]: On 29th day the animals were sacrificed by cervical dislocation, Liver and kidneys were removed, homogenized with buffer containing 0.25 M sucrose and 0.1 M Tris-Hcl buffer, pH 7.4 to prepare 10% homogenate by using in Teflon pestle and glass homogenizer and centrifuged at 600rpm for 10 min, to obtain post mitochondrial supernatant (PMT). The post mitochondrial supernatant was again centrifuged at 8000rpm for 15 min. This supernatant was used to analyze the antioxidant enzyme level in the liver.

Estimation of lipid peroxidation products (Malondialdehyde) [15]**:** The lipid peroxidation (LPO) products present in the tissue sample were estimated by the thiobarbuturic acid (TBA) method, which measured malondialdehyde (MDA) reactive product at 532 nm.

Estimation of Superoxide dismutase (SOD) [16]**:** Super oxide dismutase (SOD) activity was measured according to the method described by Marklund S and Marklund G. SOD activity was expressed as units/min/mg protein. Change in optical density per minute at 50% inhibition of pyrogallol to adenochrome transition by the enzyme was taken as one enzyme unit.

Estimation of catalase (CAT): Catalase activity was measured according to the method described by Sinha [17].

Estimation of glutathione peroxidases (GPx): Glutathione peroxidases play a major role in the scavenging the hydroxyl radical produced by free radicals and its activity was measured by the method described by Moron *et al.,* [18].

Estimation of reduced glutathione (GSH): Reduced glutathione (GSH) activity was measured according to the method described by Rotruck *et al.,* [19].

Statistical Analysis: The data's were expressed as mean ± standard error (S.E.M). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 (95% confidence limit) were considered as significance.

RESULTS:

Lipid peroxidation (LPO): A significant increase ($p<0.001$) in the liver and kidney Lipid peroxidation (LPO) was observed in STZ-NA induced diabetic rats when compared to control rats. The liver and kidney LPO levels of diabetic rats treated with AEETI (250 and 500 mg/kg p.o) was significantly ($p<0.01$) and ($p<0.001$) decreased in respective group. Whereas the glibenclamide (0.5 mg/kg p.o) treated diabetic rats showed significant ($p<0.01$) decrease when compared to STZ-NA induced diabetic rats. The results are shown in **Table 1, Fig. 1**.

TABLE 1: EFFECT OF AEETI ON LPO IN STZ-NA INDUCED DIABETIC RATS:

Group	Treatment	Dose (Kg $^{-1}$ Body Weight)	MDA (nmol/mg)	
			In Liver	In Kidney
I	Control(0.5% SCMC)	5 ml	2.56±0.42	2.28±0.33
II	Diabetic (0.5% SCMC)	5ml	4.94±0.32^{a***}	3.47±0.42^{a***}
III	Diabetic (AEETI)	250mg	3.22±0.20^{b**}	3.02±0.34^{b**}
IV	Diabetic (AEETI)	500mg	2.38±0.25^{b***}	2.21±0.17^{b***}
V	Diabetic (Glibenclamide)	0.5mg	3.16±0.24^{b**}	2.98±0.28^{b**}

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnett's t test. n=6

a- Group II is compared with Group I.

b- Group III, IV and V are compared with Group II. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$

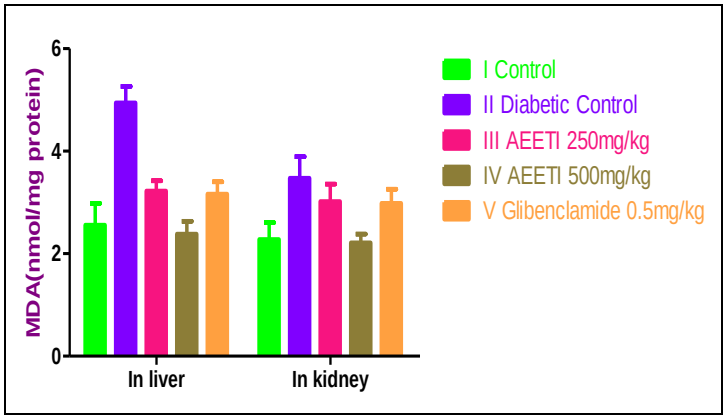

FIG 1: EFFECT OF AEETI ON LPO IN STZ-NA INDUCED DIABETIC RATS

Superoxide dismutase (SOD): A significant decrease ($p<0.001$) in the liver and kidney Superoxide dismutase (SOD) was observed in STZ-NA induced diabetic rats when compared to control rats. The liver and kidney SOD levels of diabetic rats treated with AEETI (250 and 500 mg/kg p.o) was significantly ($p<0.01$) and ($p<0.001$) increased in respective group. Whereas the glibenclamide (0.5 mg/kg p.o) treated diabetic rats showed less significant ($p<0.05$) increase when compared to STZ-NA induced diabetic rats. The results are shown in **Table 2, Fig 2**.

TABLE 2: EFFECT OF AEETI ON SOD IN STZ-NA INDUCED DIABETIC RATS

Group	Treatment	Dose (Kg $^{-1}$ Body Weight)	SOD (units/min/mg protein)	
			In Liver	In Kidney
I	Control(0.5% SCMC)	5 ml	7.16±0.21	6.45±0.11
II	Diabetic (0.5% SCMC)	5ml	3.08±0.12 ^{a***}	3.78±0.26 ^{a***}
III	Diabetic (AEETI)	250mg	5.10±0.20 ^{b**}	4.61±0.18 ^{b**}
IV	Diabetic (AEETI)	500mg	6.98±0.24 ^{b***}	5.20±0.23 ^{b***}
V	Diabetic (Glibenclamide)	0.5mg	3.93±0.21 ^{b*}	3.96±0.14 ^{b*}

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnett's t test. n=6

a- Group II is compared with Group I;

b- Group III, IV and V are compared with Group II. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$.

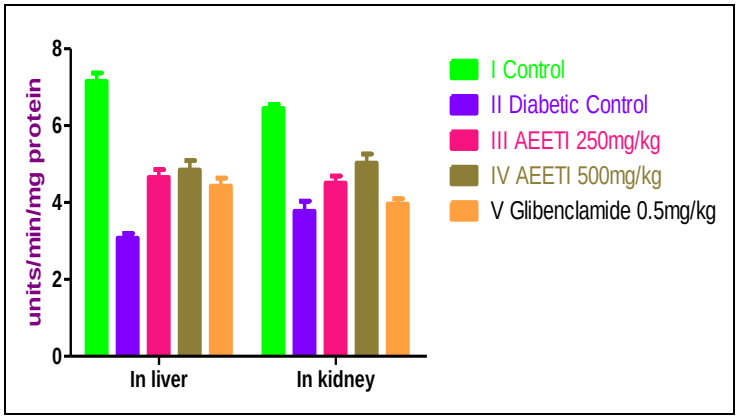

FIG. 2: EFFECT OF AEETI ON SOD IN STZ-NA INDUCED DIABETIC RATS

Catalase (CAT): A significant ($p<0.001$) decrease in the liver and kidney Catalase **(**CAT) was observed in STZ-NA induced diabetic rats when compared to control rats. The liver and kidney CAT levels of diabetic rats treated with AEETI (250 and 500 mg/kg p.o) was significantly ($p<0.01$) and ($p<0.001$) increased in respective group. Whereas the glibenclamide (0.5 mg/kg p.o) treated diabetic rats showed less significant ($p<0.01$) increase when compared to STZ-NA induced diabetic rats. The results are shown in **Table 3, Fig. 3**.

TABLE 3: EFFECT OF AEETI ON CATALASE IN STZ-NA INDUCED DIABETIC RATS

Group	Treatment	Dose (Kg $^{-1}$ Body Weight)	CAT (units/min/mg protein)	
			In Liver	In Kidney
I	Control(0.5% SCMC)	5 ml	72.51±3.50	32.53±2.19
II	Diabetic (0.5% SCMC)	5ml	51.45±4.22 ^{a***}	16.26±1.84 ^{a***}
III	Diabetic (AEETI)	250mg	66.32±3.43 ^{b**}	20.33±3.18 ^{b**}
IV	Diabetic (AEETI)	500mg	69.52±2.16 ^{b***}	23.99±1.73 ^{b***}
V	Diabetic (Glibenclamide)	0.5mg	58.17±3.84 ^{b*}	18.03±2.08 ^{b*}

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's t test. n=6

a- Group II is compared with Group I.

b- Group III, IV and V are compared with Group II. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$

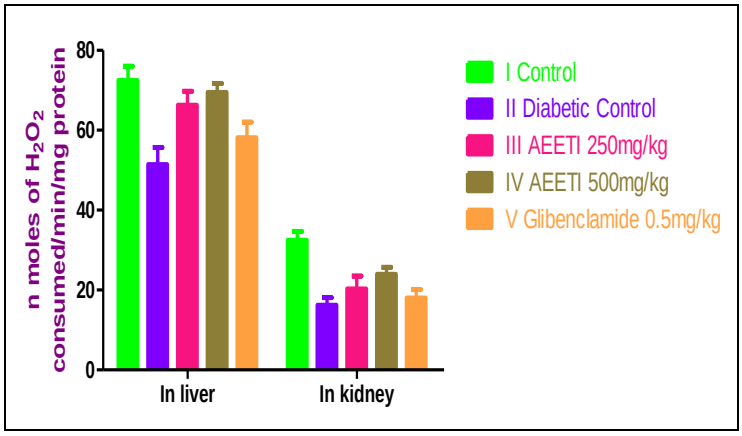

FIG. 3: EFFECT OF AEETI ON CAT IN STZ-NA INDUCED DIABETIC RATS

Glutathione Peroxidase (GPx): A significant ($p<0.001$) decrease in the liver and kidney Glutathione Peroxidase (GPx) was observed in STZ-NA induced diabetic rats when compared to control rats. The liver and kidney GPx levels of diabetic rats treated with AEETI (250 and 500 mg/kg p.o) was significantly ($p<0.01$) and ($p<0.001$) increased in respective group. Whereas the glibenclamide (0.5 mg/kg p.o) treated diabetic rats showed less significant ($p<0.05$) increase when compared to STZ-NA induced diabetic rats. The results are shown in **Table 4, Fig. 4**.

TABLE 4: EFFECT OF AEETI ON GLUTATHIONE PEROXIDASE IN STZ-NA INDUCED DIABETIC RATS

Group	Treatment	Dose (Kg^{-1} Body Weight)	GPx (units/min/mg protein)	
			In Liver	In Kidney
I	Control(0.5% SCMC)	5 ml	16.11±0.12	9.83±0.24
II	Diabetic (0.5% SCMC)	5ml	$10.29\pm0.23^{a***}$	$5.32\pm0.20^{a***}$
III	Diabetic (AEETI)	250mg	$12.17\pm0.18^{b**}$	$7.29\pm0.19^{b**}$
IV	Diabetic (AEETI)	500mg	$15.26\pm0.10^{b***}$	$8.40\pm0.12^{b***}$
V	Diabetic (Glibenclamide)	0.5mg	$10.90\pm0.09^{b*}$	$6.18\pm0.10^{b*}$

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnet's t test. n=6

a- Group II is compared with Group I.

b- Group III, IV and V are compared with Group II. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$

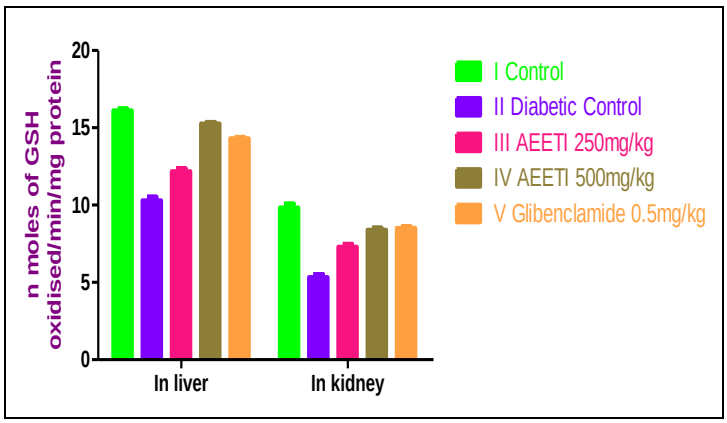

FIG. 4: EFFECT OF AEETI ON GPX IN STZ-NA INDUCED DIABETIC RATS

Reduced Glutathione (GSH): A significant ($p<0.01$) decrease in the liver and kidney Reduced Glutathione (GSH) was observed in STZ-NA induced diabetic rats when compared to control rats. The liver and kidney GSH levels of diabetic rats treated with AEETI (250 and 500 mg/kg p.o) was significantly ($p<0.01$) and ($p<0.001$) increased in respective group. Whereas the glibenclamide (0.5mg/kg p.o) treated diabetic rats showed significant ($p<0.05$) increase when compared to STZ-NA induced diabetic rats. The results are shown in **Table 5, Fig. 5**.

TABLE 5: EFFECT OF AEETI ON REDUCED GLUTATHIONE IN STZ-NA INDUCED DIABETIC RATS

Group	Treatment	Dose (Kg^{-1} Body Weight)	GSH (units/min/mg protein)	
			In Liver	In Kidney
I	Control(0.5% SCMC)	5 ml	5.22±021	4.76±0.16
II	Diabetic (0.5% SCMC)	5ml	$3.03\pm0.39^{a***}$	$2.88\pm0.10^{a***}$
III	Diabetic (AEETI)	250mg	$3.96\pm0.17^{b**}$	$3.38\pm0.23^{b**}$
IV	Diabetic (AEETI)	500mg	$4.46\pm0.13^{b***}$	$3.60\pm0.12^{b***}$
V	Diabetic (Glibenclamide)	0.5mg	$3.20\pm0.20^{b*}$	$3.12\pm0.19^{b*}$

The values are expressed as mean ± SEM. Statistical significance test was done by ANOVA followed by Dunnett's t test. n=6

a- Group II is compared with Group I.

b- Group III, IV and V are compared with Group II. $^{***}p<0.001$, $^{**}p<0.01$.

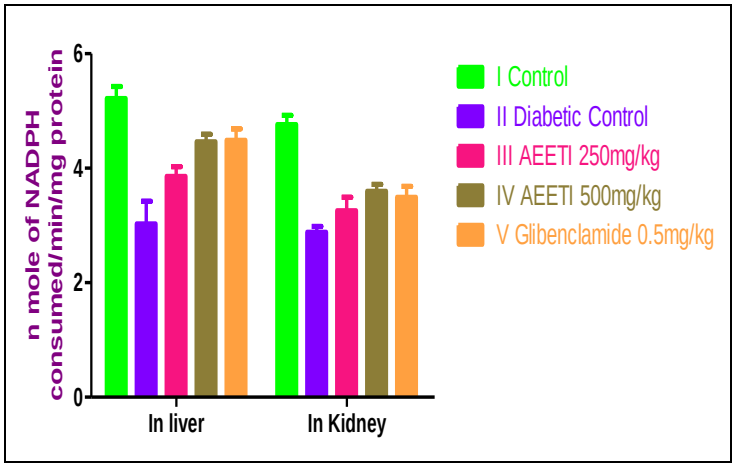

FIG. 5: EFFECT OF AEETI ON GSH IN STZ-NA INDUCED DIABETIC RATS

DISCUSSION: Oxidative stress plays a major role in the pathogenesis of diabetes mellitus by generation of oxygen free radicals as well as due to non-enzymatic glycosylation, auto-oxidation of glucose, modification in antioxidant enzyme level and formation of lipid peroxides.[20] In diabetes due to altered insulin level results in initiation of β-oxidation of fatty acid that favours the accumulation of free radicals resulting in Lipid peroxidation.

Increased Concentration of lipid peroxidation in the liver may impairs membrane functions by decreasing membrane fluidity and change the activity of membrane-bound enzyme and receptor. These lipid radical and lipid peroxide are obviously harmful to the cells in the body. Altogether there is an increase in the level of MDA in experimental diabetes. [21] In the present study diabetic control rats the level of MDA and hydrogen peroxide were high in both liver and kidney. The treatment with AEETI 250, 500 mg/kg ($p<0.01$ and $p<0.001$ respectively) and whereas the glibenclamide less significantly ($p<0.05$) decreased the MDA level which shows a protective effect of AEETI against peroxidative damage.

The enzymatic antioxidant defence mechanism contains various forms of enzymes like SOD, Catalase, glutathione peroxidase and glutathione reductase. Certain chemicals like STZ produce the free radicals thereby produce experimental induction of DM. SOD and CAT are the two major scavenging antioxidant enzymes that remove toxic free radicals in vivo. It has been reported that the reduction in SOD and CAT may leads to the accumulation of O_2^- and H_2O_2 [22]. The SOD and CAT level is significantly low in case of diabetic control rats, but treatment with AEETI 250, 500 mg/kg ($p<0.01$ and $p<0.001$ respectively) and whereas the glibenclamide less significantly ($p<0.05$) increased SOD and CAT levels in both liver and kidney which denotes the cytoprotective effect of AEETI in both liver and kidney. In addition to the level of SOD and CAT level GSH, GPx activity is also reduced in diabetic condition [23].

In the present study, the GSH and GPx activity is low in diabetic rats. Whereas the treatment with AEETI 250, 500 mg/kg ($p<0.01$ and $p<0.001$ respectively) and whereas the glibenclamide less significantly ($p<0.05$) increased the GSH and GPx activity in both liver and kidney which denotes the cytoprotective effect of AEETI in both liver and kidney.

CONCLUSION: From the above investigation we concluded that aqueous ethanolic extract of *Tragia involucrata* offers effective protection against free radicals that forms the basis for the development of diabetes and diabetic complications.

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