

In-vivo Study of Antioxidant activity of *Tephrosia spinosa* (L.f) pers in rats

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ABSTRACT

A study was under taken to evaluate the antioxidant potential of Aerial parts of *Tephrosia spinosa* (L.f) pers. The chloroform extract and methanol extract of *Tephrosia spinosa* was subjected to in vivo antioxidant screening through free radical scavenging activity using rats. Liv-52 was used as the standard. In this model study the extract showed potent antioxidant activity, thereby augmenting it into the present day system of medicine.

Keywords: Antioxidant, Chloroform extract, Methanol extract, *Tephrosia spinosa* (L.f) pers.

INTRODUCTION

The effects of free radicals on human beings are closely related to toxicity, disease and aging (Max Well., 1995). Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive oxygen species (Ros) (Sato *et al.*, 1996). The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. Due to the free radicals only majority of the diseases occur. *Tephrosia spinosa* (L.f) pers belongs to the family papilionaceae.¹ and it is a stiffy thorny shrub, known as mullukolinji commonly found in South India on dry barren lands on the coast and inland to the hills of Coimbatore, Madurai and Tirunelveli districts.² The phytochemical studies revealed the presence of flavanoids.³⁻⁴ It is used in traditional system of medicine for antirheumatic, antipyretic, indigestion, anti diarrhoeal, anti inflammatory, anthelminitic and to control excessive thirst.⁵ No systematic studies on antioxidant activity have been reported on *Tephrosia spinosa*. Hence an effort has been made to establish the antioxidant activity.

EXPERIMENTAL INVESTIGATION

a. Free radical induced by Chromium(VI)

Chromium is a naturally occurring heavy metal found commonly in the environment in the trivalent Cr (III) and hexavalent Cr (VI) forms Cr (VI) compounds have been declared as potent occupational carcinogens among workers in chrome plating, stainless steel and pigment industries. Chromium (VI) compounds are well known oxidizing agents capable of directly inducing tissue damage & and possess carcinogenic mutagenic, and teratogenic potency. Chromium (VI) compounds are easily taken up by the cells and are subsequently reduced to Cr (III) species. This reduction generates free radicals which play a major role in the adverse biological effects of this compounds.⁶

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA; Research-Lab fine chem. Industries Mumbai, India) nitro blue tertazolium chloride (NBT, Himedia laboratories Pvt.Ltd, Mumbai India). 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB Alfa Aesar, A Johnson Matthey company). Bovine serum albumin (Spectrochem Pvt. Ltd. Mumbai, India) carboxy methyl

cellulose (Research Lab, Mumbai, India) Ethylalcohol (absolute ethanol) all the chemical used were of analytical grade and purchased from standard manufacturers.

Animals

Male albino wistar rats each weighing 180-220 gms were obtained from K.M. College Pharmacy, Madurai, India. Rodent laboratory chow was access and water ad libitum, and rats were maintained on a 12 hour light/dark cycle in a temperature regulated room (20-25°C) during the experimental procedures. The animals were cared for according to the guiding principles in the care & use of animals. The experiments were approved by the institutional animal ethics committee with Registration number and date of registration 661 / 02 / CPCSEA & 19-07-2002.

CHROMIUM INGESTION AND PLANT EXTRACT OF *TEPHROSIA SPINOSA* ('TS') Administration

Rats were divided randomly into five groups of six animals each and treated for four weeks i.e. 28 days as follows:⁷

- Group I** Served as normal control group received normal saline in a dose of 100ml/kg.
- Group II** Served as Toxic Control group and was administered chromium 30mg/kg(30%v/v,1ml/100k g orally.⁸
- Group III** Served as a standard group and was administer LIV-52 in a dose of 56 mg/kg orally.
- Group IV** Served as a treatment control group and was Administered chloroform extract of *Tephrosia spinosa* (CETS) in a dose of 200mg/kg orally..
- Group V** Served as a treatment control group and was Administered methanol extracts of *Tephrosia*

spinosa (METS) in a dose of 200mg/kg.

Group III to V was given the both extract 1 hr prior to the administration of the chromium.

Biochemical Analysis

Dissection and Homogenization

On the 29th day all animal were killed by decapitation. Blood was collected and serum was separated for estimation of alanine aminotransferase (ALT) and aspartate aminotranferase (AST).⁹ The liver was rapidly excised rinsed in ice-cold saline and a 10% W/V homogenate was prepared using (0.15MKCl) potassium chloride. Centrifuged at 800 rpm for 10 min at 4°C. The supernatant obtained was used for the estimation of catalase, and lipid per oxidation. Further the homogenate was centrifuged at 1000 rpm for 20 min at 4°C and the supernatant was used for estimation of SOD and glutathione.¹⁰

Lipid Per Oxidation Assay (LPO)

Malondialdehyde (MDA), a secondary product of lipid per oxidation reacts with thiobarbitoric acid at pH 3.5. The red pigment produced was extracted in n-butanol-pyridine mixture and estimated by measuring the absorbance at 532 nm.¹¹

Superoxide dismutase activity (SOD)

Superoxide dismutase activity was assayed according to the method of kono.¹² Where in the reduction of nitro blue nitroblue tetrazolium chloride (NBT) was inhibited by superoxide dismutase and measured at 560nm spectrophotometrically. Briefly the reaction was initiated by addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of liver homogenate. The results were expressed as units per milligram of protein with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50%.

Catalase activity (CAT)

Catalase activity was assessed by the method of Luck.¹³ Where the breakdown of H₂O₂ was measured at 240nm. Briefly the assay mixture consisted of 3ml of H₂O₂ phosphate buffer (0.0125M; H₂O₂) and 0.05ml of supernatant of liver homogenate and the change in the absorbance was measured at 240nm. The enzyme activity was calculated using the mill molar extension coefficient of H₂O₂ (0.07). The results were expressed as micromole of H₂O₂ decomposed per min per milligram of protein.

Estimation of Reduced Glutathione

Reduced glutathione (GSH) in the liver was assayed according to the method of Ellman.¹⁴ Sample (0.75ml) of homogenate was precipitated with 0.75ml of 4% sulphosalicylic acid and centrifuged at 1200g for 15 min at 4°C. The assay mixture contained 0.5ml of supernatant and 4.5ml of 0.01M, DTNB. (5-5'-dithiobis (2-nitro benzoic acid)) in 0.1M, phosphate buffer (PH 8.0). The yellow colour developed was read immediately at 412nm. The results were expressed as micromole of GSH per milligram of proteins.

Determination of AST (aspartate aminotransferase)

AST (aspartate aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman'S, Frankel S.¹⁵

Determination of ALT (alanine aminotransferase)

ALT (alanine aminotransferase) from the liver in the blood serum was assayed according to the method of Reitman S, Frankel S.(10)

Statistical Analysis

The results are expressed as mean \pm SEM. Data was evaluated using one way ANOVA followed by Newman Keuls multiple range left. Probability values less than (P<0.01) were considered significant.

RESULTS AND DISCUSSION

EFFECT OF CETS AND METS ON BODY WEIGHT, FOOD AND WATER CONSUMPTION

The effect of 'CETS AND METS on body weight changes during the chromium induced oxidative stress is shown in Table No:1. Chromium feeding resulted in significant decrease in the body weight with the duration of treatment; however, in animals fed with two doses of 'CETS AND METS and chromium; there was no significant change as compared to the control group. Administration of chromium did not cause any significant change in the food and water intake.

EFFECT OF CETS AND METS ON SOD AND CATALASE LEVELS

Administration of chromium caused a significant increase (p<0.01) in the liver tissue catalase levels but did not affect SOD levels (Table No: 2).The CETS AND METS in a dose of 200mg/kg body weight was able to restore the catalase levels to that of control values

EFFECT OF CETS AND METS ON REDUCED GSH and MDA (Lipid peroxidation)

Liver tissue GSH levels were significantly decreased following the chromium treatment, where as significant increase in plasma MDA levels was observed (Table No: 2). Administration of CETS AND METS in 200mg/kg body weight, dose reverted the GSH and MDA levels to that of control values.

EFFECT OF 'CETS AND METS ON AST AND ALT LEVELS

AST and ALT levels were increased (p<0.01) in all the animals treated with chromium (Table No: 2). Administration of 200mg/kg body weight dose of CETS AND METS significantly inhibited the chromium induced increase in enzyme levels and restored to that of control values.

DISCUSSION

One of the most important early events in cell degeneration leading to necrosis is the Lipid per oxidative damage that occurs

mainly in the cell membrane. In addition, lipid peroxidation represents one of the most reaction resulting from free radicals attack on biological structures Cr(VI) and Cr(V) are both able to yield ROS.^{16,17} The majority of oxidative stress studies in rat have used TBARS as a tissue damage indicator.^{18,19}

In addition there was no study relating TEPHROSIA SPINOSA with chromium intoxication. Therefore in this study was undertaken to evaluate for the antioxidant activity against the chromium (VI) induced oxidative stress in male albino rats. The results of the present study demonstrate that the **CETS AND METS** at a concentration of 200mg/kg body weight protected the animals significantly from the chromium induced oxidative damage. Oral feeding of chromium resulted in a significant decrease in body weight. Chromium (IV) Compounds are well known oxidizing agents capable of directly inducing tissue damage and possess carcinogenic, mutagenic and teratogenic potency.²⁰ Chromium (VI) compounds are easily taken up by cells and are subsequently reduced to Cr(III) species. This reduction generates free radicals, which play major role in the adverse biological effect of these compounds.²¹ Administration of chromium significantly increases the lipid peroxidation as evident by the increase in

MDA levels. To cope with the oxidative stress, there was a significant decrease in reduced glutathione (GSH) and catalase level in the liver tissue. No significant change in the SOD activity was observed in the Chromium-treated animals and our results fall in confirmation with earlier studies.²² Besides activating the oxidative stress, Chromium also caused a marked increase in AST and ALT levels suggesting that the Chromium treatment also causes hepatic damage. Many workers have also demonstrated the hepato-toxic effect of Chromium (VI) which is mainly due to the lipid peroxidation.^{23,24} These adverse effects of Chromium (VI) could be significantly curtailed by pre treating the animals with the **CETS AND METS**.

CONCLUSION

In animals fed with both Extracts of **CETS AND METS** significant protection was observed against the chromium induced oxidative stress. The '**CETS AND METS**' inhibited the chromium induced increase in MDA levels and restore the intracellular antioxidant. Like GSH and catalase levels to that control. The **CETS AND METS** also protected the animals significantly from the hepatotoxicity induced by chromium is revealed by the decreased AST and ALT activity compared to the chromium (VI) treated animals.

Table 1: Effect of Body Weight of Normal and Experimental Animals in Each Group

Groups	Initial Body Weight	Final Body Weight
Group-I	212.5 ± 8.65	220.4 ± 6.30
Group-II	210.8 ± 6.50	160.60 ± 3.28 ^a
Group-III	215.0 ± 6.28	222.4 ± 5.20
Group-IV	206.4 ± 5.22	210.30 ± 4.60
Group-V	196.20 ± 4.16	212.64 ± 5.16

- Values are expressed as mean SEM.
- No. of animals in each group (n) = 6
- Values were found out by using one way ANOVA followed by Newman Keul's multiple range test. (a*) values were significantly different from Initial Body Weight of G₂ at (P < 0.01).

Table 2: Effect of Methanol and Chloroform Extract of Tephrosia Spinosa on Chromium Induced Free Radicals in Rats

GROUPS	SOD U/L	CATALASE	REDUCED GSH mg/dl	LIPID PEROXIDATION nmoles/ml	AST U/L	ALT U/L
Group-I	30.66±1.65	275.4±4.21	112.15±2.79	166.95±2.84	184.78±3.01	88.95±2.68
Group-II	28.41±0.93	187.41±3.25 ^a	60.16±1.30 ^a	258.28±2.34 ^a	331.86±2.66 ^a	221.90±3.13 ^a
Group-III	27.25±1.58	238.75±4.07 ^b	93.3±1.95 ^b	216.8±4.19 ^b	234.25±3.90 ^b	127.42±3.33 ^b
Group-IV	32.95±0.84	202.03±2.31 ^b	72.62±3.23 ^b	198.69±2.38 ^b	284.66±3.21 ^b	181.41±2.30 ^b
Group-V	31.79±1.31	217.45±2.11 ^b	84.21±3.05 ^b	207.44± 4.49 ^b	255.51±3.73 ^b	152.72±2.75 ^b

- Values are expressed as mean SEM.
- No. of animals in each group (n) = 6
- Values were found out by using one way ANOVA followed by Newman Keul's multiple range test.
- (^a) values were significantly different from Normal control (G₁) at (P < 0.01).
- (^b) values were significantly different from toxic group (G₂) at (P < 0.01).

Where, G₁ – Normal control group

G₂- Toxic control group

G₃- Standard group

G₄- Treatment control (Choloroform extract of Tephrosia SPINOSA,(CETS).

G₅- Treatment control (Methanol extract of Tephrosia SPINOSA,(METS).

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