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Pharmacological Evaluation and Nephroprotective effect of *Tamarindus indica* and Purslane extract against nephrotoxicity

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Abstract

The nature has provided the storehouse of remedies to cure all ailments of mankind. The traditional herbal medicines are still practiced in large part of our country mostly in tribal and rural areas. In many developing countries large section of population relies ontraditional healers, who are dependent on herbal folk medicines for their primary health care and has deep faith in it. Since the usages of these herbal medicines are increased, the issues regarding their safety, quality and efficacy in industrialized and developing countries are cropped up (1). Growing interest has also prompted researcher to screen scientifically various claims regarding properties and uses of medicinal plant materials. Presently both common consumers and health care professional seek updated, authoritative information towards safety and efficacy of any recommended medicinal plant as drug prior to its use (2). The WHO (3) has also emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards.

In present investigation we have selected three plants out of many frequently used nephron protective medicinal plants on the basis of the claims made by Unani and Ayurvedic physians (4). The literature survey revealed that the seeds of *Tamarindus indica, Purslane*

Key Words: Kidney Disease, Polyherbal Plant formulation, *Tamarindus indica, Purslane.*

Introduction

Plants are essential components of the universe. After various observations and experimentations many medicinal plants were identified as source of important medicine. Medicinal plants have been used since prehistoric period for the cure of various diseases.[1]

About 8,000 herbal remedies have been described in Ayurveda. The Rig-Veda (5000 BC) has recorded 67 medicinal plants, Yajurveda 81 species, Atharvaveda (4500-2500 BC) 290 species, Charak Samhita (700 BC) and Sushrut Samhita (200 BC) had described properties and uses of 1100 and 1270 species respectively. In compounding of drugs and these are still used in the classical formulations and in the Ayurvedic system of medicine. From time immemorial man depended on plants derived medicines, it is evident that the fascination for plants is as old as mankind itself.

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The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medical purpose and could serve as lead for the development of novel agents having good efficacy in various pathological disorders.[2] The World Health Organization (WHO) estimates that about 80% of population living in the developing countries relies on traditional medicines for their primary health care needs. In almost all the traditional system of medicines, the medicinal plants play a major role and constitute the backbone. A large body of evidence has collected to show potential of medicinal plants used in various traditional systems. In the last few years more than 13 000 plants have been studied for the various diseases and ailments all over the world. Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Most of the phytochemical beneficial effects consumed by human beings, they are used effectively treat to human disease. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediated their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as curative medicines.[4]

In general, traditional practitioners used crude drug either in fresh juice or decoction for healing various ailments. The medicinal plants belonging to established system of medicine i.e. Ayurveda, Unani and Siddha were used by the traditional folk healers. They used crude drugs in single or combination of drugs for the treatment of different diseases.

Considerable research on Pharmacognosy, Chemistry, and Pharmacology and Clinical therapeutics has been carried out on native medicinal plants. Traditional knowledge driven drug development can follow a reverse pharmacology path and reduce time and cost of development.

In Indian system of medicine several herbal remedies has been tried for the treatment of kidney failure since the time of charka and sushruta. New approaches to improve and accelerate the joint drug discovery and development process are expected to take place mainly from innovation in drug target elucidation and lead structure discovery. (5)

Extracts and metabolites of the plant particularly those from leaves and fruits possess useful pharmacological activities. The fruits are utilized as vegetable and regarded as essential ingredient in the South Indian diet.

Material and Methods

The drug *Tamarindus indica*, *Purslane* were procured from a local Indian market and were identified Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Mansarovar Global University, Bhopal.

The medicinal plant Tamarindus indica Linn (Family: Fabaceae) was selected for Nephroprotective activity based on the literature survey.

Collection and authentication of the plant

The fruit pulp of Tamarindus indica Linn was collected. The plant Tamarindus indica Linn belonging to the family of Fabaceae.

The fruit pulp of Tamarindus indica Linn were collected and shade dried at the room temperature and then cut it in to small pieces, which was used for the extraction for further studies.

MACERATION

Fresh fruit pulps of Tamarindus indica Linn were cut into small pieces, seeds were removed and air dried. The dried pieces of Tamarindus indica Linn fruit pulp, weighing 100 g, were soaked in 500 ml of 95% ethanol in a round bottom flask for About 24 hours. (6)

EXTRACTION

Extracting values of crude drug are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.(7)

Ethanolic extract

Solvent - Ethanol

The process of extraction was done by reflux condensation method using soxhlet apparatus at 60-80 ºC for 9 hours. The extract was

concentrated by distillation apparatus till a syrupy consistency was obtained. Finally, the extract was put in a china dish and evaporated at40-60 ºC temperature in a water bath, 22 gms of semisolid extract was obtained.

Table-1: Nature and colour of Ethanolic extract of Tamarindus indica Linn

S. No	Name of the plant extract	Part used
1.	Tamarindus indica Linn	Fruit pulp

Preliminary phytochemical screening

The Ethanolic extract of the fruit pulp of Tamarindus indica Linn was subjected to a preliminary phytochemical screening to identify the active chemical constituents.

Test solution

The Ethanolic extract of the fruit pulp of Tamarindus indica Linn was taken and dissolved in distilled water.

TEST FOR CARBOHYDRATE

A small quantity of the extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrate and glycosides.

Molisch's test

Few drops of Molisch's reagent were added to an aqueous solution of each extract followed by vigorous shaking. Thereafter, 1.0 ml of conc. H_2SO_4 was added carefully by sliding down the walls of the tube gently to form two layers. There is an appearance of brown ring separating the solution into two layers, it indicate the presence of carbohydrate.

Fehling's test

To 1.0 ml of aqueous solution of each extract was added 3.0 ml of a mixtureof equal volumes of Fehling's solutions A and B and boiled in a water bath at about40 °C for 2 min. A brick red colour at the bottom of the test tube was an indication ofthe presence of reducing sugar.

Anthrone test

2 mg of the extracts was shaken with 10ml water, filtered concentrated. To this 2ml of anthrone reagent solution was added. Formation of green or blue colour indicated presence of carbohydrates. (7)

TEST FOR GLYCOSIDES

Tests for glycosides were performed as follows:

(i) To 0.1 g of each extract in a test tube was added 5.0 ml of water and the mixture heated in a water bath at 100 °C for 2 min. The mixture was filtered through a Whatman No. 1 filter paper. A mixture of Fehling's solutions A and B were added to the filtrate until it became alkaline: followed by heating for 2 min.

(ii) The above procedure was repeated, except that 5.0 ml of dilute sulphuric acid was added to 0.1g of the extract instead of water: and the quantity of precipitate formed was noted.

(iii) About 0.1 g of each extract was put into a stoppered conical flash in which was suspended a strip of sodium picrate paper. The flask was warmed gently for about an hour at 37 °C and allowed to stand. The test paper was examined for any change in colour. (8)

Legal's and Borntrager's tests

Another portion of the extract was hydrolysed with hydrochloric acid for a few hours on a water bath and then hydrolysate was subjected to Legal's and Borntrager's tests to detect the presence of different glycosides.

Legal' test

To the hydrolysate, 1ml of pyridine and a few drops of sodium nitroprussside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearence of pink to red colour showed the presence of glycosides. (9)

Borntrager's test

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal of the diluted ammonia solution was added. Ammonia layer acquired pink colour, showing the presence of glycosides.

TEST FOR TANNINS

Small quantity of the extract was taken in water and test for the presence of tannins was carried out with the following reagents.

Ferric chloride test

A 5%solution of ferric chloride in 90% of alcohol was prepared. Few drops of this solution was added in to a little of the above filtrate. Dark green or deep blue colour is obtained, it indicate the presence of tannins.

Lead acetate test

A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate is obtained, presence of tannins.

Potassium dichromate test

If on an addition of solution of potassium dichromate in test filtrate, dark colour is developed, tannins are present.

TEST FOR SAPONINS

Presence of saponins was determined by their frothing property as well as capacity to form emulsion with oils. (i) For the frothing test, about 5 mg of extract was shaken vigorously with sodium bicarbonate and examined for frothing; (ii) For the emulsification test, 2 drops of olive oil was added to 5.0 mL of aqueous solution of the extract in a test, shaken vigorously and observed for formation of an emulsion. The control was without extract but water and olive oil.

Foam test

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15minutes. There is formation of one layer of foam, whichshowed the presence of saponins.

TEST FOR FLAVANOIDS

With aqueous sodium hydroxide solution

Small quantity of the extract was dissolved in aqueous sodium hydroxide.

Yellow colour was produced which indicated the presence of flavones. (10)

With conc. sulphuric acid

To the small portion of extract, concentrated sulphuric acid was added. Yellow to orange colour was produced which indicated the presence of flavones.

Shinoda's test

Small quantity of the extract was dissolved in ethanol. To them pieces of magnesium were added followed by conc. hydrochloric acid drop wise added and heated. Appearance of magenta colour showed the presence of flavonoids.

TEST FOR ANTHROQUINONES

Approximately 0.1 g of the extract was mixed with 5.0 ml of chloroform and agitated for 5.0 min. The solution was filtered and equal volume of ammonia was added to the filtrate and agitated again. A brick red colour in the upper aqueous layer indicates the presence of free anthroquinones.

TEST FOR ALKALOIDS Dragendroff's test

To the extract Dragendroff's reagent (potassium bismuth iodine solution) was added. A reddish brown precipitate was produced, which indicated the presence of alkaloids.

Mayer's test

To the extract Mayer's reagent (potassium mercuric iodine solution) was added. A reddish brown precipitate was produced, which indicated the presence of alkaloids.

Wagner's test

To the extract Wagner's reagent (iodine-potassium iodide solution) was added. A reddish brown precipitate was produced, which indicated the presence of alkaloids.

Hagner's test

To the extract Hagner's reagent (Saturated solution of picric acid) was added.

A yellow precipitate was produced, which indicated the presence of alkaloids. (2)

Tannic acid test

To the extract tannic acid solution was added. A buff colour precipitate was produced, which indicated the presence of alkaloids.

TEST FOR PHYTOSTEROL AND TERPENES

A 1.0 g weight of the extract was mixed with 5.0 ml of 95% ethanol and then filtered. The filtrate was evaporated to dryness and the residue re-dissolved in 5.0 mlof anhydrous chloroform and then filtered. The latter filtrate was divided into two portions for the following tests: (2)

Liebermann-Burchard Test

The first portion was mixed with 1 ml of acetic anhydride followed by the addition of 1.0 ml of concentrated Sulfuric acid gently down the side of the test tubeto form a layer underneath. The formation of a reddish violet colour at the junction of the two liquids and a green colour in the chloroform layer would indicate the presence of terpenes.

Salowski's Test

To the second portion of the solution was added 2.0 ml of concentrated Sulfuric acid carefully down the side of the tube so that the sulfuric acid formed a layer. No reddish brown colour at the interface would indicate the absence of sterols.

The results of phytochemical screening were shown in Table no.5

Pharmacological Evaluation

ACUTE TOXICITY STUDY

Whenever an investigator administers a chemical substance to a biologicalsystem, different types of interactions can occur and a series of dose-related responses result. In most cases these responses are desired and useful, but thereare a number of other effects which are not advantageous. These may or may not be harmful to the patients. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of new drug involve acute, sub- acute and chronic toxicity. Acute toxicity is involved in estimation of LD50 (the dose has proved to be lethal (causing death) to 50% of the tested group of animals). (11)

Determination of oral toxicity is usually an initial screening step in the assessment and the evaluation of the toxic characteristics of all compounds. This article reviews the methods of so far utilized for the determination of median lethal dose (LD50) and the new changes which would be made. This has to go through the entire process of validation with different categories of substances before its final acceptance by regulatory bodies.

Organisation for Economic co-operation and Development (OECD) regulates guidelines for oral acute toxicity study. It is an international organisation which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing. To determine the acute oral toxicity OECD frames thefollowing guideline methods.

OECD 401 – Acute Oral Toxicity

OECD 420 – Acute Oral Toxicity: Fixed Dose procedure OECD 423 –Acute Oral Toxicity: Acute Toxic Classic methodOECD 425 – Acute Oral Toxicity: Up and own Procedure

In the present study the acute oral toxicity of Spermacoce ocymoides Burn f was carried out according to OECD 423 guideline (Acute Oral Toxicity: acute Toxic Classic Method).

ACUTE ORAL TOXICITY

Acute oral toxicity refers to those adverse effects that occur followingoral administration of a single dose of a substance or multiple doses given within 24 hours.

LD_{50} (median lethal oral dose)

LD_{50} (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

PRINCIPLE

It is based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a step wise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound- related mortality of the animals dosedat one step will determine the next step, i.e.;

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose level.

SELECTION OF ANIMAL SPECIES

The preferred rodent species was the rat. Normally females were used. Females were generally slightly more sensitive. Healthy young adult animals of commonly used laboratory strains were employed. Females were nulliparous and non-pregnant. Each animal, at the commencement of it's dosing, were between 8 to 12 weeks old. (2)

ADMINISTRATION OF DOSES

The test substance was administered in a single dose by gavages using a oral feeding needle. Animals were fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water was withheld for 3-4 hours). Following the period of fasting, the animals were weighed and the test substance administered. After the substance has been administered, food was withheld for a further 3-4 hours in rats.

OBSERVATION

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation was not fixed rigidly. It was determined by the toxic reactions, time of onset and length of recovery period and extended when considered necessary. The times at which signs of toxicity appeared and disappeared were important, when toxic signs were to be delayed. All observations were systematically recorded with individual records being maintained for each animal. (12)

Fixation of doses of the extract

An acute oral toxicity study was carried out according to OECD guidelines. No adverse effect was reported or mortality in albino wister rats up to 2000mg/kg p.o. ofethanolic extracts of Tamarindus indica Linn.

Therefore, the maximum tolerated dose 200mg/kg & 400mg/kg was chosen for further studies.

Animals

Albino wistar rats of either sex (150-200 gm) were procured from the animal house . Prior to the experiment the rats were housed in a clean polypropylene cage (6 rats/cages) for a period of 7 days under temperature (25-30^{0}c), relative humidity (45-55%).

The Institutional Animal Ethics Committee approved the experimental protocol and the conditions in the animal house approved by Committee for Supervision on Experiments on Animals. The study was conducted in accordance with IAEC guidelines.

Drugs and chemicals

All the drugs, chemicals and reagents were procured from S.D Fine chemicals, Mumbai, India. All chemicals and reagents used were of analytical reagent.

Experimental protocol

The Nephroprotective activity was tested on five groups of albino wistar rats of either sex, each group consisting of six animals.

Group-I : Served as normal control received 0.5 % DMSO (Dimethylsulphoxide) ; for 15 days.

Group-II : Served as Nephrotoxic control, received vehicle (0.5% DMSO);for 15 days.

Group-III : Received the standard Nephroprotective drug, (Lipoic acid(50mg/kg; p.o)) dissolved in DMSO for 15 days.

Group-IV : Received ethanolic extract of Tamarindus indica Linn (200mg/kg; p.o) dissolved in DMSO for 15 days.

Group-V : Received ethanolic extract of Tamarindus indica Linn (400mg/kg;p.o) dissolved in DMSO for 15 days.

On the 10th day 2 hours after the administration of standard Nephroprotectivedrug (Lipoic acid) and Tamarindus indica (200 & 400 mg/kg) II-V groups received cisplatin (7.5mg/kg; i.p).

Blood collection techniques used in the present study

At the end of the experimental period, ie on the 15th day animals were sacrificed under mild ether anesthesia. The blood was collected by retro-orbital vein puncture using a fine capillary to an anticoagulant tube and allowed to stand for 30 min at 37°C and then centrifuged to separate the serum to evaluate the biochemical markers. (13)

Preparation of kidney homogenate

The kidney was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl). A portion of the kidney was homogenized in chilled Tris-HClbuffer (0.025 M, pH 7.4) using a homogenizer. The homogenate obtained was centrifuged at 5000 rpm for 10 minutes, supernatant was collected and used for various biochemical assays.

ANALYIS OF GENERAL PARAMETERS

Estimation of urine volume

The animals are kept in separate metabolic cages for 24 hours. Each rat urine volume are taken after 24 hours. The food wastes and fecel matters are removed from the urine. And the volume of urine is measured by using measuring cylinder.

Estimation of Body weight

At the end of the experiment, each group of the animals were kept individually in the cages. Remove the food and water, and each animal

are individually weighed and the weight were recorded.

ANALYIS OF SERUM BIOCHEMICAL PARAMETERS

Estimation of Serum Creatinine

Five test tubes were labelled as A, B, C, D and E. Where A&B is taken as standard, C &D taken as test and the E where taken as blank. Into E (blank), 2 ml of distilled water, into C&D (test), 0.5 ml serum and 1.5 ml of water, into A&B (standard), 1.5 ml of water and 0.5 ml of creatinine standard (3mg/dl) were pipette out. 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added in all the five test tubes.

Reagents

Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)

Creatinine working standard for serum (3mg/dl): dilute 10 ml of stock andmake the volume up to 500 ml with water.

Serum samples.NaOH (2.5 M), Picric acid.

Table-3

	Standard (A, B)	Test (C,D)	Blank (E)
Serum	-	0.5 ml	-
Distilled water	1.5 ml	1.5ml	2 ml
Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml
NaOH (2.5 M)	0.4 ml	0.4 ml	0.4 ml

Mix well

Add 0.4 ml of 2.5 M NaOH

Allow to stand for 20 minutes

$$\text{Serum creatinine} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times \text{Concentration of std}$$

Read the absorbance against the blank at 520 nm

Estimation of Serum Blood urea nitrogen (BUN) [234]

The blood urea was estimated by Berthelot method (Fawcett and Scott, 1960) using the commercially available kit (Kamineni Life Sciences Pvt. Ltd. Hyderabad, India). 1000 µl of working reagent-I containing urease reagent, and a mixture of salicylate, hypochlorite and nitroprusside was added to 10 µl of serum, 10 µl of standard urea (40 mg/dl) and 10 µl of purified water to prepare test, standard and blank, respectively. All the test tubes were mixed well and incubated at 37 °C for5 min. Then 1000 µl of reagent-II containing alkaline buffer, was added to all the test tubes, which were incubated at 37 °C for 5 min. Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia thus released reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield indo phenol, a blue- green coloured compound. The intensity of the colour produced is directly proportional to the concentration of urea in the sample and is measured spectrophotometrically at 578 nm. The blood urea was calculated using the following formula:

ANALYIS OF URINE BIOCHEMICAL PARAMETERS

Estimation of Creatinine clearance

Five test tubes were labelled as A, B, C, D and E. Where A&B is taken as standard, C&D taken as test and the E where taken as blank. Into E (blank), 2 ml of distilled water, into C&D (test), 0.5 ml urine and 1.5 ml of water, into A&B (standard),ml of water and 0.5 ml of creatinine standard (3mg/dl) were pipette out. 6 ml of picric acid and 0.4 ml of sodium hydroxide (NaOH) (2.5M) were added in all the fivetest tubes.

Creatinine stock standard: 150 mg creatinine in 100 ml water (1.5 mg/ml)Creatinine working standard for urine (0.75 mg/dl): dilute 50 ml of stock and bring the volume up to 200 ml with water.

Urine samples., NaOH (2.5 M), Picric acid.

Table-2

	Standard A, B	Test C,D	Blank E
Urine	-	0.5 ml	-
Distilled water	1.5 ml	1.5ml	2 ml

Standard for serum	0.5 ml	-	-
Picric acid	6 ml	6 ml	6 ml
NaOH (2.5 M)	0.4 ml	0.4 ml	0.4 ml

mix well
Add 0.4 ml of 2.5 M NaOH
Allow to stand for 20 minutes
Read the absorbance against the blank at 520 nm

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine}}{\text{Serum creatinine}} \times \text{volume of urine ml/min/1.73 m}^2$$

ANALYIS OF OXIDATIVE STRESS PARAMETERS

Estimation of malondialdehyde (MDA)

Lipid peroxidation (LPO) was assayed by the method of in which the malondialdehyde (MDA) released served as the index of LPO. The extent of LPO in the hepatic tissue was assayed by measuring one of the end products of this process, the thiobarbituric acid-reactive substances (TBARS). As 99% TBARS is malondialdehyde (MDA), thus this assay is based on the reaction of 1 molecule of MDA with 2 molecules of TBARS at low pH (2- 3) and at a temperature of 95°C for 60 min. The resultant pink chromogen can be detected spectrophonometrically at532 nm.

Reagents: Standard: 1, 1, 3, 3-tetra ethoxypropane (TEP), 8.1% Sodium dodecyl sulphate (SDS), 20%Acetic acid, 0.8%Thiobarbituric acid (TBA), 15:1 v/v n-butanol: pyridine mixture

Procedure

To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95.8ºC for 60 min using glass ball as a condenser. After cooling, 1 ml of water and 5 ml of n-butanol: pyridine (15:1 v/v)mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10min, the organic layer was taken and its absorbance was measured at 532 nm. The level of MDA was expressed as nmoles of mg of tissue. (2)

ANALYSIS OF ENZYMATIC ANTIOXIDANTS PARAMETERS

Estimation of superoxide dismutase (SOD) [237]

This enzyme catalyzes the dismutation of superoxide anion ($O2^{-}$) to hydrogen peroxide and molecular oxygen in the following manner

The enzyme activity was assayed by the method of Misra and Fridovich, 1972

Reagents

0.1 M Carbonate-bicarbonate buffer; pH 10.2.
0.6 mM EDTA solution
1.8 mM Epinephrine (prepared in situ)
Absolute ethanol.
Chloroform

Procedure

0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, added 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate-bicarbonate (pH 10.2) buffer. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured. The unit for superoxide dismutase (SOD) is nmoles/mg of protein. (2)

Estimation of Catalase (CAT). [238]

This enzyme catalyzes conversion of hydrogen peroxide into water and molecular oxygen.

The enzyme activity was assayed by the method of Sinha ,1972.

Reagents

Dichromate-acetic acid reagent: 5% potassium dichromate in water wasmixed with glacial acetic acid in the ratio of 1:3 (v/v).
0.01 M Phosphate buffer; pH 7.0.
0.2M Hydrogen peroxide

Procedure

0.1 ml of the tissue homogenate was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0) pre-warmed to 37°C, 0.4 ml of distilled water and the mixture was incubated at 37°C. The reaction was initiated by the addition of 0.5 ml of 0.2 M hydrogen peroxide and the reaction mixture was incubated at 37°C for one minute. The reaction was terminated by the addition of 2 ml of dichromate-acetic acid reagent after15, 30, 45, and 60 seconds. Standard hydrogen peroxide in the range of 4-20 µl/ moles were taken and treated

in the same manner. All the tubes were heated in a boiling water bath for 10 minutes, cooled and the green colour that developed was read at 590 nm against blank containing all components except the enzyme. Catalase activity was expressed in U/mg protein. (14)

Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase activity was determined according to the method of Hafemann et al. (1974). The activity of GPx was determined by measuring the decrease in GSH Content after incubating the sample in the presence of H_2O_2 and NaN_3.

Reagents

5 mM GSH

25 mM H_2O_2

25 mM NaN_3

Phosphate Buffer (0.05 mM, pH 7)

1.65 % HPO_{32}

0.4 M Na_2HPO_4

1 mM DTNB

Procedure

Tissue homogenate (approximately 0.5 mg protein) was incubated with 0.1 mlof 5mM GSH, 0.1 ml of 1. 25 mM H2O2, 0.1ml of 25 mM NaN3 and phosphate buffer (0.05 mM, pH 7) in a total volume of 2.5 ml at 37oC for 10 min. The reaction was stopped by adding 2 ml of 1.65 % HPO32- and the reaction mixture was centrifugedat 1500 rpm for 10 min. 2 ml of the supernatant was mixed with 2 ml 0.4 M Na2HPO4 and 1ml of 1mM DTNB. The absorbance of the yellow colored complexwas measured at 412 nm after incubation for 10 min at 37oC against distilled water. A sample without the tissue homogenate processed in the same way was kept as non enzymatic reaction. (15)

Results and Discussion

Phytochemical screening

Different chemical tests were performed for phytochemical screening of methanolic extract of ***Tamarindus indica*** together with tests for alkaloids, glycosides, tannins, sugar, carbohydrates, saponins, proteins, amino acids, resins, lipids/fats, phenolic compounds and flavonoids as discussed by the reported method. The screening of methanolic extract exposed the presence of flavonoids, terpenoids, saponins, phenolic compounds, proteins and amino acids. Phytochemical analysis revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Tannins bind to proline rich protein and interfere with protein synthesis. The ability of flavonoids is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. They also are effective antioxidant and show strong anticancer activities. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Glycosides are known to lower the blood pressure according to many reports. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit .

S.No	Parameters	% w/w (Mean ± SD*)
1	LOD	4.55±0.05
2	Moisture content	3.20±0.55
3	**Ash value**	
	Total ash	5.45±0.05
	Water soluble ash	2.41±0.04
	Acid insoluble ash	2.54±0.04
4	**pH**	
	10 % solution	5.3±0.20
	1 % solution	5.3±0.15
5	**Cold extraction**	
	Petroleum ether	2.03±0.05
	Chloroform	5.15±0.35
	Methanol	12.53±0.14
	Aqueous	15.51±0.45
5	**Hot extraction**	

	Petroleum ether	3.31±0.13
	Chloroform	11.14±0.51
	Methanol	32.05±0.51
	Aqueous	35.53±0.24
5	**Successive extraction**	
	Petroleum ether	2.55±0.12
	Chloroform	5.15±0.15
	Methanol	25.43±0.15

*Standard deviation

Physicochemical evaluation of *Purslane*

All physicochemical parameters were repeated for three times and are reported in by means of SD (standard deviation). The Karl Fischer method was used for fortitude of water to confirm the product superiority. The results confirmed that the loss on drying was 5.45 % and moisture content was 4.05 %. Inorganic materials, like carbonates, silicates, oxalates and phosphates can be validate by the grit of total ash. Ash value designate about adulteration, value and purity of the plant material and their products. The acid insoluble ash is responsible for silica while high acid insoluble ash mainly for the contamination with sandy materials. Inorganic elements can be calculated by means of water-soluble ash.

The total ash in *Purslane* sample was found 5.55% w/w, whereas acid insoluble and water soluble ash were 3.52% w/w and 3.15±0.05% w/w, respectively. It was considered that the drug was found acidic in nature at the pH of 1.0 and 10% i.e. 5.5 and 5.5 respectively. The yields measure the quantity of phytoconstituents in an extract with their compatible. Fixed oil, resin and volatile substances are come out in the petroleum ether extract, while at 105^0C all the volatile substances are evaporated though fixed oil, resins remain. The high yield was set up in methanol extract (15.15% w/w) when hot extraction was conceded where lowest amount of yield was set up in petroleum ether (0.55% w/w) when cold extraction was conceded (Table 3).

Table 3. Results of physicochemical evaluation of Purslane (n=3)

S.No	Parameters	% w/w(Mean ± SD*)
1	LOD	5.45±0.02
2	Moisture content	4.05±0.54
3	**Ash values**	
	Total ash	5.55±0.04
	Water soluble ash	3.15±0.05
	Acid insoluble ash	3.52±0.01
4	**pH**	
	10 % solution	5.5±0.10
	1 % solution	5.5±0.15
5	**Cold extraction**	
	Petroleum ether	0.55±0.13
	Chloroform	5.55±0.41
	Methanol	10.55±0.35
	Aqueous	15.45±0.25
5	**Hot extraction**	
	Petroleum ether	2.55±0.15
	Chloroform	5.35±0.45
	Methanol	15.15±0.45
	Aqueous	25.53±0.34
5	**Successive extraction**	
	Petroleum ether	2.15±0.23
	Chloroform	5.25±0.51
	Methanol	15.0±0.51

***In-vitro* antioxidant and nephroprotective activity**

In-vitro *antioxidant activity by reversed phase HPLC*

The electron donation ability of natural products can be measured by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. Results of this study suggest that the plant extract rich in phenolics or

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flavonoids constituents are capable of donating hydrogen to a free radical to scavenge the potential damage (Siddique *etal*., 2010). The developed HPLC method used for screening antioxidant activity was found to be simple, sensitive and specific for DPPH free radical scavenging activity. Method allowing short run time and rapid determination of radical scavenging activity of different plants extract. Radical Scavenging (%) was calculated using the formula quoted in experimental method.

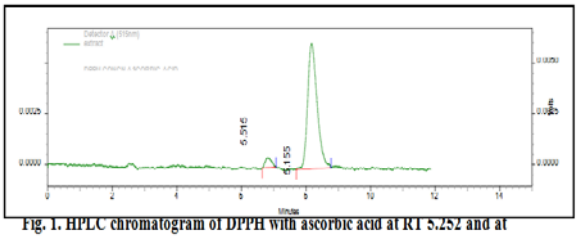

Fig. 1. HPLC chromatogram of DPPH with ascorbic acid at RT 5.252 and at wavelengthof 515 nm; mobile phase; methanol: water (50: 20 *v/v*); flow rate 1 mL min^{-1}; injection volume 20 µl

DPPH radical scavenging activity of aqueous extract of* Tamarindus indica *(AEMP)

The radical scavenging activity of standard ascorbic acid and AEMP were determined by HPLC method at 515nm (Fig. 35-40). A reduction in peak area revealed comparable radical scavenging activity of ascorbic acid at 20µg mL^{-1}(IC_{50} 15.55) and AEMP at 30µgmL^{-1} (IC_{50} 22.54)

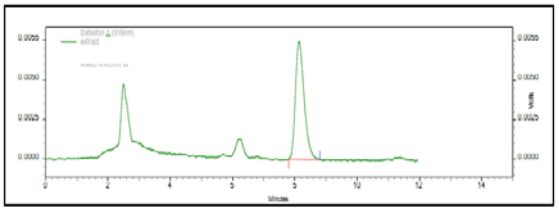

Fig. 2. HPLC chromatogram of DPPH with AEMP at RT 5.133 and at wavelength 515 nm; mobile phase; methanol : water (50 : 20 v/v); flow rate 1 mLmin^{-1}; injection volume 20 µl

DPPH radical scavenging activity of aqueous extract of *Purslane* (AEPO)

The radical scavenging activity of standard ascorbic acid and AEPO were determined by HPLC method at 515nm (Fig. 35-41). A reduction in peak area revealed comparable radical scavenging activity of ascorbic acid at 20µg mL^{-1}(IC_{50} 15.55) and AEPO at 40 µgmL^{-1} (IC_{50} 25.55) (Table 52).

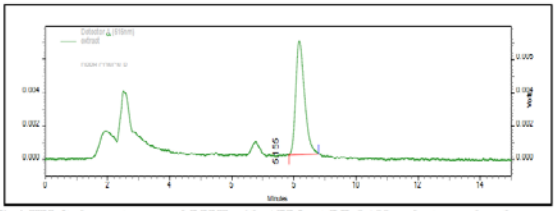

Fig.3.HPLC chromatogram of DPPH with AEPO at RT 5.155 and at wavelength 515nm; mobile phase; methanol: water (50:20 *v/v*); flow rate 1mLmin^{-1}; injection volume 20µl

In-vivo *antioxidant and nephroprotective activity*

Effect of aqueous extract of *Tamarindus indica* on blood urea concentrations in cisplatin inducednephrotoxicity in rats

The cisplatin showed definite signs of nephrotoxicity and marked renal dysfunction, as compared to the control groups, evidenced by elevation of the blood urea level. The AEMP at the dose of 500 mgkg^{-1} body weight was found to normalize the raised blood urea and bring about marked recovery in kidneys tissues. Blood urea concentrations were significantly increased ($p<0.05$) in the toxicant (CP) group (Group II) of animals compared to the normal control group signifying the induction of severe nephrotoxicity. Treatment with the AEMP showed significant ($p<0.01$) decrease in the (Group IV, V and VI) concentrations of serum urea compared to the toxicant group. The results showed significant inhibition of raised blood urea level in test drugs. AEMP at a dose of 500 mgKg^{-1} significantly inhibited the rise of blood urea level. The mean score were 33.50 ± 1.35 in normal control group, 55.55 ± 1.52 in toxicant group, 35.50 ± 1.51 in group V AEMP (500 mgKg^{-1}), 53.51 ± 0.55 in group IV AEMP (400 mgKg^{-1}) and 53.55 ± 1.51 in group III AEMP (200 mgKg^{-1}) (Fig 45, Table 53). The overall percent reversal of toxicity inhibition of AEMP was 51.51% in group V at higher dose of 500 mgKg^{-1} with respect to normal control (100%) and toxic control (0%). The results are shown in(Table 55).

The cisplatin showed definite signs of nephrotoxicity and marked renal dysfunction, as compared to the control groups, evidenced by elevation of uric acid concentration. The AEMP at the dose of 500 mgkg^{-1} body weight was found to normalize the raised uric acid concentration and bring about marked recovery in kidneys tissues.

Uric acid concentrations were significantly increased ($p<0.05$) in the toxicant (CP) group (Group II) of animals compared to the normal control indicating the induction of severe nephrotoxicity. Treatment with the AEMP showed significant ($p<0.01$) decrease in the (Group IV, V and VI) concentrations of uric acid compared to the toxicant group. The results showed significant inhibition of raised uric acid level in test drugs. AEMP at a dose of 500 $mgKg^{-1}$ significantly inhibited the rise of uric acid level. The mean score were 1.55 ± 0.055 in normal control group,
4.13 ± 0.055 in toxicant group, 2.41 ± 0.052 in group V AEMP (500 $mgKg^{-1}$), 3.05 ± 0.035 in group IV AEMP (400 $mgKg^{-1}$) and 3.55 ± 0.05 in group IIIAEMP (200$mgKg^{-1}$) (Fig 45, Table 53). The overall percent reversal of toxicity inhibition of AEMP was 50.0% in group V at higher dose of 500 $mgKg^{-1}$ with respect to normal control (100%) and toxic control (0%). The results are shown in (Table 55).

Effect of aqueous extract of *Tamarindus indica* on serum creatinine concentrations in cisplatin induced nephrotoxicity in rats

The cisplatin showed definite signs of nephrotoxicity and marked renal dysfunction, as compared to the control groups, evidenced by elevation of serum creatinine concentration. The AEMP at the dose of 500 $mgkg^{-1}$ body weight was found to normalize the raised serum creatinine concentration and bring about marked recovery in kidneys tissues. Serum creatinine concentrations were significantly increased ($p<0.05$) in the toxicant (CP) group (Group II) of animals compared to the normal control indicating the induction of severe nephrotoxicity. Treatments with the AEMP showed significant ($p<0.01$) decrease in the (Group IV, V and VI) concentrations of serum creatinine compared to the toxicant group. The results showed significant inhibition of raised serum creatinine level in test drugs. AEMP at a dose of 500 $mgKg^{-1}$ significantly inhibited the rise of serum creatinine level. The mean score were 0.25 ± 0.042 in normal control group, 3.45 ± 0.054 in toxicant group, 0.45 ± 0.051 in group V AEMP (500 $mgKg^{-1}$), 1.24 ± 0.055 in group IV AEMP (400 $mgKg^{-1}$) and 3.35 ± 0.055 in group III AEMP (200 $mgKg^{-1}$) (Fig. 50, Table 53). The overall percent reversal of toxicity inhibition of AEMP was 53.10 % in group V at higher dose of 500 $mgKg^{-1}$ with respect to normal control (100%) and toxic control (0%).

Table 53. Results of biochemical estimation in serum of different group of *Tamarindus indica* *(n=5)*

Groups	lood urea ($mgdl^{-1}$)	Uric acid ($mgdl^{-1}$)	Serum creatinine ($mgdl^{-1}$)
Normal control (group I)	33.50 ± 1.35	1.55 ±0.055	0.25 ± 0.042
Toxicant–cisplatin (5 $mgkg^{-1}$b.w, ip)(group II)	55.55 ± 1.52##	4.13 ±0.055##	3.45 ± 0.054##
Tamarindus indica lower dose (200 $mgkg^{-1}$)(group III)	53.55 ± 1.51*	3.55 ±0.055*	3.35 ± 0.055*
Tamarindus indica medium dose (400 $mgkg^{-1}$)(group IV)	53.51 ± 0.55**	3.05 ±0.035**	1.24 ± 0.055**
Tamarindus indica higher dose (500 $mgkg^{-1}$) (group V)	35.50 ± 1.51**	2.41 ±0.052**	0.45 ± 0.051**
M. pruriens dose (500 $mgkg^{-1}$)(group VI)	35.55 ± 1.25**	2.55 ±0.045**	0.41 ± 0.053**

Values are mean ± S.E; One-way Analysis of Variance (ANOVA) followed by Dunnett test## $P<0.01$ vs group I (Normal control), * $P<0.05$, **$P<0.01$ vs Toxic control

Effect of aqueous extract *Purslane* on blood urea concentration in gentamicin induced nephrotoxicity in rats

Blood urea concentration was significantly increased ($p<0.05$) in the toxicant (GM) group (Group II) of animals compared to the normal control group signifying the induction of severe nephrotoxicity. Treatment with the AEPO showed significant ($p< 0.01$) decrease in the (Group IV, V and VI) concentrations of serum urea compared to the toxicant group. The results showed significant inhibition of raised blood urea level in test drugs. AEPO at a dose of 500 $mgKg^{-1}$ significantly inhibited the rise of blood urea level. The mean score were 23.23 ± 1.45in normal control group, 55.45 ± 1.55 in toxicant group, 44.53 ± 1.42 in

group V AEPO (500 $mgKg^{-1}$), 45.35 ± 1.04 in group IV AEPO (400 $mgKg^{-1}$) and 53.5 ± 1.55 in group III AEPO (200 $mgKg^{-1}$) (Fig. 50, Table.55). The overall percent reversal of toxicity inhibition of AEPO was 50.55% in group V at higher dose of 500 $mgKg^{-1}$ with respect to normal control (100%) and toxic control (0%). The results are shown in (Table.55).

Effect of aqueous extract *Purslane* on uric acid concentration in gentamicin induced nephrotoxicity in rats

Uric acid concentration was significantly increased ($p<0.05$) in the toxicant (GM) group (Group II) of animals compared to the normal control indicating the induction of severe nephrotoxicity. Treatment with the AEPO showed significant ($p<0.01$) decrease in the (Group IV, V and VI) concentrations of uric acid compared to the toxicant group. The results showed significant inhibition of raised uric acid level in test drugs. AEPO at a dose of 500 $mgKg^{-1}$ significantly inhibited the rise of uric acid level. The mean score were 2.05 ± 0.05 in normal control group, 3.54 ± 0.05 in toxicant group, 3.05 ± 0.04 in group V AEPO (500 $mgKg^{-1}$), 3.14 ± 0.05 in group IV AEPO (400 $mgKg^{-1}$) and 3.55 ± 0.05 in group III AEPO (200 $mgKg^{-1}$) (Fig.51, Table.55). The overall percent reversal of toxicity inhibition of AEPO was 44.31% in group V at higher dose of 500 $mgKg^{-1}$ with respect to normal control (100%) and toxic control (0%). The results are shown in (Table.55).

Effect of aqueous extract of *Purslane* on serum creatinine concentration in gentamicin induced nephrotoxicity in rats

Serum creatinine concentration was significantly increased ($p<0.05$) in the toxicant (GM) group (Group II) of animals compared to the normal control indicating the induction of severe nephrotoxicity. Treatments with the AEPO showed significant ($p<0.01$) decrease in the (Group IV, V and VI) concentrations of serum creatinine compared to the toxicant group. The results showed significant inhibition of raised serum creatinine level in test drugs. AEPO at a dose of 500 $mgKg^{-1}$ significantly inhibited the rise of serum creatinine level. The mean score were 0.33 ± 0.05 in normal control group, 4.45 ± 0.05 in toxicant group, 0.52 ± 0.05 in group V AEPO (500 $mgKg^{-1}$), 1.50 ± 0.05 in group IV AEPO (400 $mgKg^{-1}$) and 4.35 ± 0.05 in group III AEPO (200 $mgKg^{-1}$) (Fig.52,Table.55). The overall percent reversal of toxicity inhibition of AEPO was 52.55% in group V at higher dose of 500 $mgKg^{-1}$ with respect to normal control (100%) and toxic control (0%).

Table: 5. Results of biochemical estimation in serum of different group of *Purslane* *(n=5)*

Group number	lood urea ($mgdl^{-1}$)	Uric acid ($mgdl^{-1}$)	Serum creatinine ($mgdl^{-1}$)
Normal control (group I)	23.23 ± 1.45	2.05 ± 0.52	0.33 ± 0.05
Toxicant– gentamicin (40 $mgkg^{-1}$b.w, s.c) (group II)	55.45 ± $1.55^{\#\#}$	3.54 ± $0.05^{\#\#}$	4.45 ±$0.05^{\#\#}$
Portulaca lower dose (200 $mgkg^{-1}$) (group III)	53.50 ± 1.55^{ns}	3.55 ± 0.05^{ns}	4.35 ± 0.05^{ns}
Portulaca medium dose (400 $mgkg^{-1}$) (group VI)	45.35 ± 1.04^{**}	3.14 ± 0.05^{**}	1.50 ± 0.05^{**}
Portulaca higher dose (500 $mgkg^{-1}$) (group V)	44.53 ± 1.42^{**}	3.05 ± 0.01^{**}	0.52 ± 0.05^{**}
Portulaca prophylectic dose (500 $mgkg^{-1}$) (group VI)	40.55 ± 1.55^{**}	3.21 ± 0.11^{**}	0.55 ± 0.05^{**}

Values are mean ± S.E; One-way Analysis of Variance (ANOVA) followed by Dunnett test# # P<0.01 vs group I (Normal control), * P<0.05, **P<0.01 vs Toxic control

Conclusion

Overall *Tamarindus indica* offered significant nephroprotection against cisplatin and gentamicin induced nephrotoxicity and showed significant reduction in blood urea, uric acid and serum creatinine and TBARS levels as well increase in SOD, GSH and Catalase levels compared to toxicant group.

Significant percent reversal of biochemical parameters on treatment with different doses of *Tamarindus indica* against cisplatin (5 mg kg^{-1}

b.w, ip) and gentamicin (40 mg kg^{-1} b.w, sc) in respect to normal control (100%) and toxic control (0%) was observed and compared with percent reversal of higher dose of ***Tamarindus indica*** and DPPH free radical scavenging efficacy.

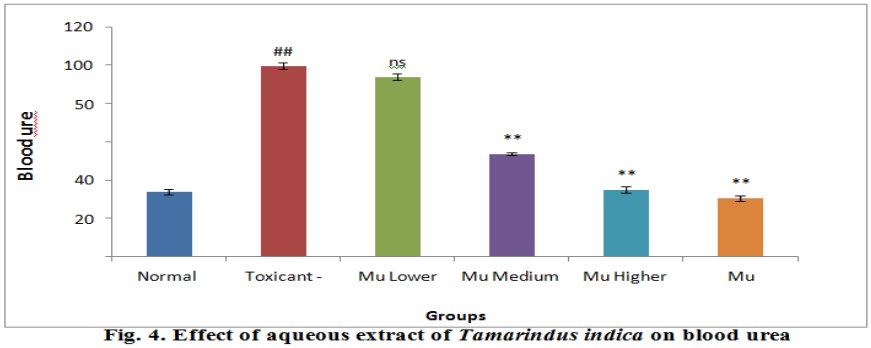

Fig. 4. Effect of aqueous extract of *Tamarindus indica* on blood urea
Effect of aqueous extract of *Tamarindus indica* on uric acid concentrations in cisplatin induced nephrotoxicity in rats

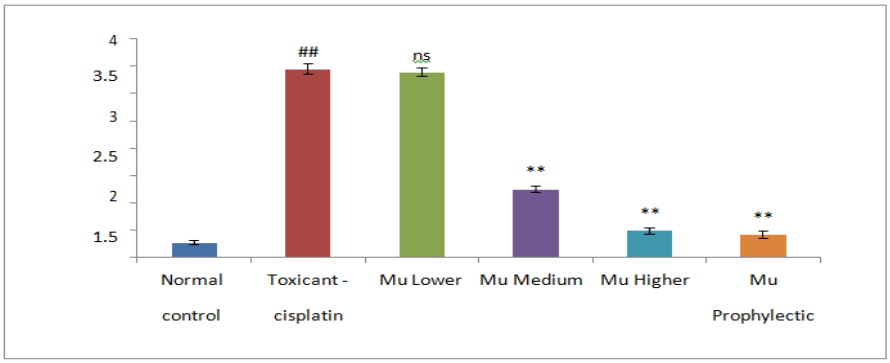

Fig. 5. Effect of aqueous extract of *Tamarindus indica* on serum creatinine

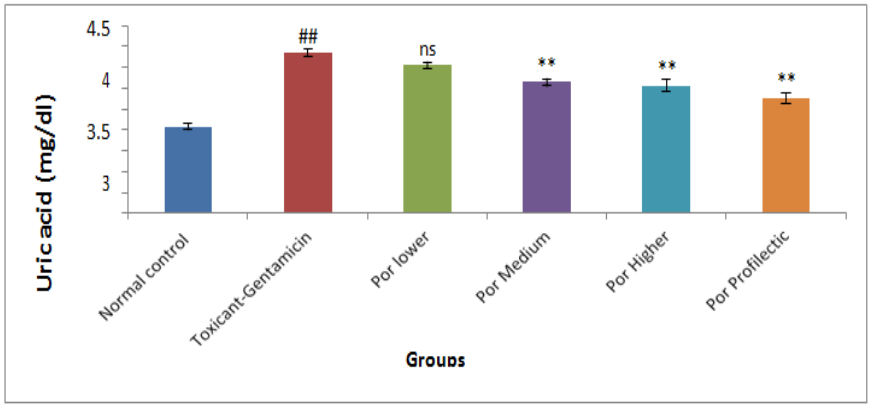

Fig. 6.Effect of aqueous extract of *Purslane* on uric acid

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