Evalution Of Nephroprotector Activity Of Hydroalcoholic Extract Of Tamarindus Flowers On Cisplatin Induced Nephrotoxicity In Albino Rats

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Abstract: Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. *Tamarindus indica L. (T. indica*), belongs to the family Leguminosae (Fabaceae). It have been therapeutic acitivities like anti inflammatory, analgesic, anticancer[4], Flower : appetizing , urinary discharges , bad odour in perspiration[5], this research article is the study of evalution of nephroprotector activity.

Introduction:

Medicinal plants: Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations [1] The distribution analysis of the medicinal plants shows that they are distributed across diverse habitats and landscape elements. Nearly about 70% of the medicinal plants in India are found in tropical forests in Eastern and western Ghats, Chota Nagpur plateau, Aravalis, Vindhyas and the Himalayas. Among the Himalayas, Kashmir Himalayan region is nestled within the Northwestern folds of the recently designated global biodiversity hotspot of the Himalayas [2].

Tamarindus *[Tamarindus indica L. (T. indica*)], belongs to the family Leguminosae (Fabaceae).

Geographical distribution and uses: Tamarind tree is found especially in the Indian subcontinent, Africa. It is a large tree 12-18 m. high, branches spreading , glabrous Leaves: 5-12.5cm long, rachis slender channeled, stipulus linear ,cadcous .

Leaflets: Subsessile, 10-20 pairs, tolerably closely set on the rachis, 8-30 by 5-8 mm. oblong,obtuse, glabrous reticulately veined .

Flowers: Few –flowered racemes at the end of the branchlets , Pedicil 6-10 mm . Long slenderarticulate below the calyx, glabrous, bracts concave, 6-8 mm. long, enclosing the buds. Cadcous ;bracteoles small . Calyx 1.3 cm. long ; tubes narrowly turbinate , 4 mm. long ; segments 8 mm.long , sub equal oblong , some what oblique , obtuse or subacute . Petals 3 (an upper and 2lateral), 1 cm. long , sub equal obovate-, oblong yellowish with pink strip. Stamens 3 fertile,connate nearly half their length; filament pubescent at the base ; anther oblong . Ovary stalked;8-12 or more style pubescent, equalling stamens. Puds 7.5 -20 cm. long by 2.5 cm. broad and about 1 cm. thick, slightly curved , subcompressed , scurfy. Seeds 3-12 obovate-oblong , truncateat the ends , 1.6 by 0.8 cm. , compressed with shallow oblong pit on the each of the flat faces,smooth , brown sining [3], according to phyto vhemical evalution it consist of phenolic compounds, carbohydrates, Proteins, vitaminA, C, E, K. Thiamine. It have been therapeutic acitivities like anti inflammatory, analgesic, anticancer[4], Flower : appetizing , urinary discharges , bad odour in perspiration[5],this research article is the study of evalution of nephroprotector activity.

Nephrotoxicity is the toxicity in the kidneys. It is a poisonous effect of some substance, both toxic chemicals and medications, on renal function. Nephrotoxicity is an intrinisic adverse effects of certain anti cancer drugs. Anticancer drugs have therapeutic effect but it may also produce nephrotoxicity.

Cisplatin is also called as diaminechloride is extensively used to treat oncological disorders, particularly bladder cancer, metastatic ovarian cancer, and metastatic testicular cancer. Testicular, ovarian, bladder, head and neck, esophegeal, small and non-small cell lung, breast, cervical, stomach and prostate cancers. And it is also used to treat Hodgkin's and non-hodgkin's lymphomas, neuroblastoma, sarcomas, multiple myeloma, melanoma, and mesothelioma.

Cisplatin is called as "the penicillin of cancer" because it is used so widely and it was the first big chemotherapy drug. The most serious and usually dose limiting toxicity of cisplatin is renal. Experimental cisplatin induced nephrotoxicity was first reported in 1971.

Materials and methods

Chemicals:

All the chemicals used were of analytical grade and procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India

Preparation of plant extract

Tamarindus Flowers were collected from nidamarru feilds , near Mangalagiri, Guntur, and cleaned by using distilled water, and shade dried , after complete dry ,the flowers were powdered , and ethanolic extract was prepared by using soxalation.

Animals:

Albino Wistar rats were weighing (150-170 g)) of either sex were procured from mahaveer enterprises, Hyderabad. They were kept in departmental animal house in well cross ventilated room at 24 hrs with light and dark cycles of 12 h for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was given ad libitum.

Acute oral toxicity studies :Acute toxicity study was performed according to OECD guidelines No. 423[17]. Swiss albino mice of either sex were divided into six groups with six animals each. SXE was administered orally as a single dose to mice at different dose levels of 250, 500, 1 000, 1 500 and 2 000 mg/kg b.w. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 d.

Tratment protocol: Thirty wistar rats (150-170 g) were divided into 5 groups of 6 animals each.

Group A: control rats (CON) that received normal saline (i.p.) and 0.5% carboxymethyl cellulose (CMC) (p.o.) for 9days.

Group B: Cisplatin treated rats that received cisplatin 60mg/kg b.w (i.p.) on 5^{th} day as a single dose and 0.5% CMC (p.o.)

Group C: receive TE 200mg/kgb.w for 9 d.and cisplatin 60mg/kg b.w.on 5^{th} day as a single dose

Group D: treated rats that received TE 200mg/kgb.w for 8 d. cisplatin 60mg/kg b.w.on 5th day as a single dose

On ninth day the animals were kept in metabolic cages and urine was collected, on tenth day

Collection of blood and isolation of serum

Blood was collected from animals by puncturing retro-orbital venous sinus and allowed to clot at 37 °C for 40 min and centrifuged at 3000 rpm for 20 min. The serum samples was taken in a clean mini centrifuge tubes and kept refrigerated until further used.

Preparation of tissue homogenate

72 h after cisplatin treatment all rats were weighed and sacrificed for isolation of kidney. After isolation, kidney samples were immediately weighed and frozen in dry ice and stored at −80 °C until further analysis. Kidney to body weight ratio was calculated. All the enzyme assays were performed on next day of kidney isolation. The Kidney were minced into small pieces and homogenized in ice cold phosphate buffer saline (0.05 M, pH 7.0) to obtain a 10% homogenate. The homogenate was centrifuged at 17000*g* for 60 min at 4 °C and the supernatant was used for assay of protein, Catalase, SOD, GPx, GR and MDA level.

Assessment of nephroprotective activity:

Hematological analysis

The Renal function test was determined by assaying blood urea nitrogen (BUN) and serum creatinine, BUN and creatinine concentration were measured by Urea assay Kit and Creatinine assay kit respectively

Estimation of free radical scavenging enzyme

The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GR) in kidney tissue were assayed according to the methods reported by various groups (Masayasu and Hiroshi [7], Paglia and Valentine [8], Cohen et al. [9] and Carlberg and Mannervik [10] .

Estimation of total protein

Total protein content in tissue was determined by the method of Lowry et al. [11] using bovine serum albumin (BSA) as standard.

Statistical analysis

All the data was analyzed by one- way analysis of variance (ANOVA) using Minitab 17 software. Values $P \leq 0.05$ were considered as statistically significant. Values are represented as Mean ± SEM, for four rats in each group.

Results:

Phytochemical Constituents Tamirindus flower extract:

Phyto constituent	PRESENT/ABSENT
Carbohydrate and glycosides	+
Oils	+
Proteins and amino acids	+
Saponin	+
Phenolic compounds	+

Phytosterols	+
Alkaloids	+
Flavonoids	+
Tannins	+

Estimation Of Serum Parameters & Urinary Parameters

S.NO	GROUP	BUN	SC	UTP	CLcr
1.	Control	22.08+-0.80	0.6+-0.05	07.6+-0.15	18.80+-1.6
2.	Cisplatin	42.05+-1.10	2.25+-0.20	20.1+-0.12	0.510+-1.5
3.	Extract(low dose)	27.01+-0.10	1.90+-0.10	08.4+-0.01	13.05+-1.2
4.	extract(high dose)	29.05+-0.05	1.14+-0.05	0.64+-0.15	11.00+-0.2

Estimation Of Tissue Homogination Parameters:

S.NO	GROUP	GSH	LOP	CAT
1.	I	145.12+-0.1	11.12+-0.1	01.25+-0.12
2.	II	72.15+-2.3	15.12+-0.1	08.10+-0.57
3.	III	85.42+-2.7	12.12+-0.5	12.05+-1.3
4.	IV	90.10+-1.90	12.0+-0.0	14.0+-1.00

Each value represents mean ± SEM from six animals in each group.

P,0.05 when compared with normal group.

Conclusion:

When the high dose (400mg/kg) were administered to the rats, no toxicity was observed.So the ethanolic extract of roots of C. gladiata have no side effects and also it has the protective effect against cisplatin induced nephrotoxicity.The nephroprotector activity in rats was further more concluded by viewing the results of the following parameters.

BUN: In the cisplatin induced rats the BUN levels are vigorously increased and when the extracts of T.E was administered the levels are lowered and has attained the normal level in case of (400 mg/kg) dose.

SC: In the cisplatin induced rats the SC levels are vigorously increased and when the extacts of T.E was administrated the levels are lowered significantly.

UTP: In the cisplatin induced rats the UTP levels are vigorously increased and when the extracts of T.E was administered the levels are significantly lowered.

CLcr: In the cisplatin induced rats are the CLcr levels are lowered down and when the extracts of T.E was administered the levels are significantly increased.

LPO: In the Cisplatin induced rats the LPO levels are increased than the normal level and when the extracts of T.E was administered the levels are significantly decreased.

GSH: In cisplatin induced rats the GSH levels are decreased than the normal level and when the extracts of T.E was administered the levels are significantly increased.

CAT: In the cisplatin induced rats the CAT levels are decreased than the normal level and when the extracts of T.E was administered the levels are significantly increased.

Hence by viewing the above results we can conclude that T.M attenuates the nephrotoxicity of cisplatin in rats and confirm the antioxidant potential of tamirndus.

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