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ISSN: 2278-4772

Ayurpharm Int J Ayur Alli Sci., Vol. 6, No. 7 (2017) Pages 130 – 148

Research Article

HYPOURICAEMIC ACTIVITY OF GANDHAPRASAARINI (*Paederia foetida* Linn.) – AN EXPERIMENTAL EVALUATION

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Received: 20-06-2017; Revised: 18-07-2017; Accepted: 29-07-2017

Abstract

Sedentary lifestyle leads to various health issues like hyperuricemia which is a precursor for many disorders like Gouty arthritis, Uremia, Renal calculi etc. Gandhaprasaarini is one of the drugs in Ayurveda possessing sothahara property and is indicated for vatarakta. As per recent research, this drug is found to have uricosuric property. The aim of this study is to investigate the efficacy of Gandhaprasaarini (*Paederia foetida* Linn.) on hyperuricemia induced Wistar albino rats (in vivo). 2×TED group showed significant reduction in serum urea level immediately after PNO_3 induction ($p<0.05$) and non-significant reduction in uric acid level after 24hrs of PNO_3 when compared to Control and Standard groups. Histopathology reveals that, in TED x 2 dose (Gandhaprasaarini) administered group, good protection was observed – cell infiltration, tubular dilatation were mild and not seen in all the rats.The results suggest that Gandhaprasaarini is proved effective in hyperuricemia condition. This study provides evidence for the use of this formulation with xanthine oxidase inhibitors clinically.

Keywords: Ayurveda; Hyperuricemia; Gouty arthritis; Gandhaprasaarini; Wistar albino rats.

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Cite This Article

Nikhil Ajaykumar, Chandrakanth Bhat, Hariprasad Shetty. Hypouricaemic activity of Gandhaprasaarini (*Paederia foetida* Linn.) – An experimental evaluation. Ayurpharm Int J Ayur Alli Sci. 2017;6(7):130-148.

INTRODUCTION

Uricaemia refers to an excess of uric acid or urates in the blood. Uric acid is a heterocyclic compound of Carbon, Nitrogen, Oxygen, and Hydrogen with the formula $C_5H_4N_4O_3$. It forms ions and salts known as urates and acid urates. Uric acid is a product of the metabolic breakdown of purine nucleotides. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions like diabetes and formation of kidney stones. Normal uric acid levels are 2.4-6.0 mg/dl (female) and 3.4-7.0 mg/dl (male). Hyperuricaemia is a potentially harmful condition which favors precipitation of uric acid crystals in joints and tissues leading to complications such as gout, nephrolithiasis and chronic nephropathy.[1] It is also a risk factor for cardiovascular diseases.

Treatment principles for hyperuricaemia include usage of NSAIDs, uricosuric drugs or xanthine oxidase inhibitors like Probenecid. Allopurinol is the most widely used antihyperuricaemic agent.[2] Major metabolite of allopurinol is oxypurinol and both allopurinol and oxypurinol are competitive inhibitors of the enzyme xanthine oxidase. The usual dose is 200-300mg/day.

Allopurinol is well tolerated by most patients, but hypersensitivity reactions may develop, which can be severe or fatal. Hepatotoxicity, bone marrow depression, intestinal nephritis is rare but are serious adverse effects of allopurinol. Hence, there is need for a medicine which won't produce any side effects and at the same time should reduce the raised level of uric acid in blood.

The present drug of study, *Paederia foetida* Linn. has no much information available regarding its pharmacological activity, hence attains importance in experimental pharmacology. Previous studies shows the dual actions of *Paederia scandens* extract as a hypouricaemic agent: xanthine oxidase (XO) inhibitory activity and uricosuric effect.[3] Taking this factor in to consideration, an attempt is being made in the present study to evaluate Gandhaprasaarini kashaya for anti-hyperuricaemic activity in rats.[4]

MATERIALS AND METHODS

Test drug

The whole plant Gandhaprasaarini (*Paederia foetida* Linn.) was collected from college premises of Muniyal Institute of Ayurveda Medical Sciences, Manipal after obtaining authentication from Gopalakrishna Bhat K, (Retd.) Professor of Botany, Poornaprajna College, Udupi. Plant was cleaned properly, dried in shade and coarsely powdered and sieved using sieve No.180 as per WHO standards for medicinal plant materials. Drug was stored in clean air tight container.

Chemicals used are Formalin (10%); Ether; Normal Saline

Selection of animals

Healthy male Wistar albino rats (30 no.) of approximately 7 to 8 weeks with weights in the range of 180-220g were taken from the animal house of S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. Reg. No. 558/02/CPCSEA.

The animals were housed in standard laboratory condition of light and dark cycle of 7am to 7pm, temperature of 25^0c and 30-60% relative humidity in well ventilated polypropylene cages. Animals were provided with normal mouse chow (Sai Durga Food and Feeds, Bangalore, India) and water ad libitum. The animals were randomly selected, marked with the help of picric acid to permit individual identification, and kept in their cages for 7 days prior to the start of dosing to allow for acclimatization to the laboratory conditions. The experiment was conducted after obtaining the permission from the

institutional ethics committee in accordance with the guide line formulated by CPCSEA.

Inclusion criteria

Healthy male Wistar Albino rats weighing 180g - 220g was taken for the study.

Exclusion criteria

Rats subjected to other experiments, Female albino rats, Rats with other pathological conditions were excluded.

Drugs Used: Standard drug: **Allopurinol** [Brand Name: ALLOFINE: Batch Number: T-1512127]

Allopurinol belongs to a group of medicines called enzyme inhibitors (Xanthine oxidase). Allopurinol is used in the treatment of gout[5] and complication during the treatment of cancer. It reduces the amount of uric acid in the body by blocking one of the processes that makes it.

Common side effects include Hypersensitivity, Fever, Muscle pain, cardiovascular diseases etc.Chemical formula of Allopurinol is $C_5H_4N_4O$.

Hyperuricaemic agent: Potassium Oxonate[6] **[**Brand Name: Alfa Aesar, Ward hill, Canada Batch Number: LOT: K05Z045].

It is the potassium salt of oxonate, an enzyme inhibitor that modulates 5-fluorouracil toxicity. Molecular formula of Potassium Oxonate is $C_4H_2KN_3O_4$. It should be kept in refrigerator to maintain temperature.

Form of drug administration

The trial drugs are administered in the form of kashaya. (Decoction)

Preparation of Drug

Group 1 & 2

100mg of CMC is taken, added with 20ml of distilled water and mixed well, (1ml /100g).

Group 3 (Allopurinol tablet)

Allopurinol tablet weighing 300mg were finely powdered and added 75mg CMC, made into suspension with adding of 15ml distilled water and administered according to 1ml/100g body weight.

Group 4 & 5

Gandhaprasaarini kashayam

Gandhaprasaarini kashayam is prepared based on classical reference at Muniyal Institute of Ayurveda Medical Sciences, Manipal.

Test Group 4: 1part (7.5g) of the drug is added with 16 parts (120ml) of water, boiled and reduced to 1/8th part (15ml). Decoction is allowed to self-cool and used for administration.

Test Group 5: 1part (15g) of the drug is added with 16parts (240ml) of water, boiled and then reduced to 1/8th part (30ml). This decoction is allowed to self-cool and used for administration.

Potassium oxonate: It was taken 450mg, added with 15ml of distilled water and mixed well and administered intraperitonially.

1. **Dose fixation**

Based on Paget and Barnes formula (1964) involving body surface area constant of rat and human dose (H.D).[7]

i.e., H.D $\times$ 0.018 $\times$ 5 /Kg body weight
100 ml $\times$ 0.018 $\times$ 5 = 9.0ml/Kg body weight
200ml $\times$ 0.018 $\times$ 5 = 18.0 ml/Kg body weight (TED$\times$2)

Test drug 1 (Gandhaprasaarini kashayam TED): Human dose = 100ml

Rat dose = 100×0.018×5=9ml/kg body wt = 0.009ml/gm

Test drug 2 (Gandhaprasaarini kashayam 2×TED): Human dose = 100ml

Rat dose = 2×100×0.018×5=18ml/kg body wt= 0.018ml/gm

Grouping of animals

Group 1: Normal Control –administered with CMC
Group 2: Positive control/disease control induced with Potassium oxonate
Group 3: Reference Standard administered with Allopurinol
Group 4: Gandhaprasaarini kashayam TED+ Hyper urecaemia induced with Potassium oxonate
Group 5: Gandhaprasaarini kashayam 2×TED+ Hyper urecaemia induced with Potassium oxonate

2. Route of drug administration:

The test drugs, reference standard, toxicant drug were administered according to the body weight by oral route with the help of rat feeding needle attached to syringe.

Experimental procedure [8][9][10][11]

Animals were divided into five groups containing six animals in each group. First group served as normal control administered orally with 0.5% CMC and received regular rat food and drinking water ad libitum. Second group administered with normal water and diet for 7 consecutive days and served as disease control. Third group administered with Allopurinol 180mg/kg orally for 7 consecutive days and served as reference standard. Fourth group administered with test drug Gandhaprasaarini kashaya at therapeutic dose (9ml/kg– 0.009ml/g), dose given for 7consecutive days and served as test group one. Fifth group administered with test drug Gandhaprasaarini kashaya at double the therapeutic dose (18ml /kg –0.018ml/g), given orally for 7 consecutive days and served as test group two.

On 7th day for group 2,3,4,5 after an hour of administration of specific drug, potassium oxonate 450mg/kg is given intraperitonially and for investigation all rats were anaesthetized with diethyl ether and blood was collected from retro-orbital plexuses after 3hrs and 24hrs for estimation of serum biochemical parameters.

After collecting blood, animals are kept in the separate metabolic cage for 24 hours for urine collection. On 8th day urine samples are collected and kept for analysis. Then animals were sacrificed by over dose of diethyl ether. The abdomen was opened by midline incision and kidney was dissected out carefully and cleaned off the extraneous tissue. Kidney was weighed and one kidney of each animal was transferred to 10% formalin solution and sent for histo-pathological studies. The measurement of uric acid concentration in blood plasma rats is performed by using enzymatic method.[12]

Parameters studied

a) Ponderal changes: Body weight on initial day and before sacrifice, weight of kidney; the weight of kidney was expressed in terms of absolute value.

b) Urine analysis: On the 8th day animals are kept in the separate metabolic cage for 24 hours urine collection. On the 9th day urine samples were collected and kept for analysis like urine microscopy and presence of crystals in urine.

c) Serum parameters: Blood samples were collected from retro orbital plexus of each animals in plain tube with clot activator (red coloured cap) and kept for centrifuge

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ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 6, No. 7 (2017) Pages 130 – 148

at 2000 rpm for 8 minutes. After that the serum was separated, collected and analyzed for parameters like urea, creatinine, and uric acid in auto analyzer.

d) Procedure for parameters:

Urine parameters

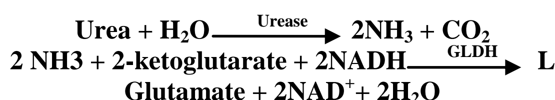
Urine microscopy: Uric acid crystals in urine were analyzed by the microscopic examination. The microscopic examination of urine includes observations of epithelial cells, white blood cells, red blood cells, crystals, casts, bacteria etc. This examination is otherwise called urine sediment examination. Urine sediment is obtained by centrifuging 10ml of urine at 3000 rpm for 5 minutes. Discard the clear supernatant fluid, place a drop of the sediment on a glass slide and cover it with cover slip. Examine first under low power and then under high power field of the microscope.

Serum parameters

Serum Urea: Proteins cannot be stored in human body, so excess should be broken down. Amino acids which form the components of proteins break down to give ammonia. This is toxic & so through a series of chemical reactions (urea cycle) nontoxic urea is produced & excreted in the urine.Elevated levels are seen during increased protein breakdown, dehydration, vomiting, and diarrhoea. It is also seen in renal disorders like glomerular nephritis, chronic nephritis &nephritic syndrome. Decreased levels are found in liver failure and pregnancy.

Principle:

Enzymatic determination of urea according to the following reaction.



Reagent composition

Urea U.V (S.L) R1 2 × 24 mL/2 × 40 mL/2 × 100 mL

Tris Buffer (pH 7.60): 100 mmol/L
A.D.P : 0.7 mmol/L
A-ketoglutarate : 9.0 mmol/L
Urease : > 6500 U/L
GLDH : > 1100 U/L

Urea U.V (S.L) R22 × 6 mL/2 × 10 mL/2 × 25 mL

NADH : 0.25 mmol/L
2- Oxoglutarate : 5 mmol/L

Urea U.V STANDRAD 1 × 4 mL

Standard concentration for urea: 50mg/dL

Preparation and stability of work reagent

Mix 4 volume of reagent 1 (R1) with 1 volume of reagent 2 (R2)

Working reagent is stable for 30 days at 2-8^{0}c.

Laboratory procedure

	Standard	Sample
Working reagent	1000µL	1000µL
Standard	10µL	-
Sample	-	10µL

Mix and read the optical density (T_1) 30 seconds after the sample or standard addition. Take second reading (T_2) exactly 60 seconds after the first reading.

Calculation

Urea Conc. (mg/ dL) = (T_1 – T_2) of sample× 50
(T_1 – T_2) of standard

Urea BUN Conc. (mg/ dL) = (T_1 – T_2) of sample× 23.4
(T_1 – T_2) of standard

Serum Creatinine

It is formed in muscles from phosphocreatine. It is important form of energy being a store of

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ISSN: 2278-4772
Ayurpharm Int J Ayur Alli Sci., Vol. 6, No. 7 (2017) Pages 130 – 148

high-energy phosphate. Creatinine determinations have one advantage over urea determination that it is not affected by a high protein diet. Serum creatinine is more specific & sensitive indicator of renal function. Simultaneous estimations of serum urea & creatinine provide better information. Serum urea nitrogen, creatinine ratio is > 15 in pre renal failure, and < 10 in renal failure. Decreased levels are found in muscle dystrophy.

Principle

Creatinine reacts with picric acid to produce a colored compound, creatinine alkaline picrate. The change in absorbance is proportional to the creatinine concentration.

Reagent composition

Creatinine dye reagent: 2 × 50 mL
Picric acid : 8.73 mmol/ L
Surfactant
Creatinine base reagent: 2 × 50 mL
Sodium hydroxide: 300 mmol/ L
Sodium Phosphate: 25 mmol/ L
Creatinine standard: 1 × 4 mL
Creatinine standard concentration: 2 mg/dL
Preparation and Stability of working reagent
Mix 1 volume of Reagent 1 (R1) with 1 volume of Reagent 2 (R2).

Laboratory procedure

	Standard	Sample
Working reagent	1000µL	1000µL
Standard	100µL	-
Sample	-	100µL
Mix and read the optical density (T_1) 60 seconds after the sample or standard addition. Exactly 60 seconds after the first reading take second reading (T_2)		

Calculation

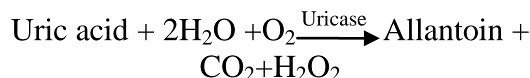
Creatinine Conc. (mg/ dL) = (T_2 – T_1) of sample× 2
(T_2 – T_1) of standard

Serum Uric acid

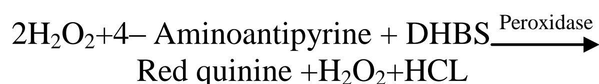
Uric acid is the end product of purine metabolism. Uric acid is excreted by the kidneys. Increased levels are found in gout, arthritis, impaired renal functions and starvation. Decreased levels are found in yellow atrophy of the liver, Wilson's disease and Fanconis syndrome.

Principle

Enzymatic determination of uric acid according to the following reactions.



$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$



$$2H_2O_2 + 4\text{– Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{Red quinine} + H_2O_2 + \text{HCL}$$

DHBS = 3, 5 - Dichloro-2-Hydroxybenzenesulfonic acid.

Reagent Composition

Uric acid R1 2×53mL/4×50mL
Phosphate buffer (pH 7.5) 100mmol/L
DHBS 2mmol/L

Uric acid R2 5×20mL/4×50mL
4- Aminoantipyrine> 0.23mmol/L
Peroxidase > 660 U/L
Uricase> 60U/L

Uric acid standard 1×4 mL
Uric acid standard concentration 6mg/dL

Preparation and stability of working reagent

Reconstitute the reagent 2 (R2) with the volume of reagent 1(R1) indicated on the vial label. Working reagent is stable for 30 days at 2 - 8°c.

Laboratory procedure

	Blank	Standard	Sample
Working reagent	1000μL	1000μL	1000μL
Standard	-	20μL	-
Sample	-	-	20μL
Mix and incubate 5 min. at 37^0c. Measure absorbance of sample and standard against the reagent blank.			

Calculation

Uric acid Con. (mg/dL) = Absorbance of sample× 6

Absorbance of standard

Histopathology of kidney

The organ like kidney was transferred to 10% formalin and sent to a commercial laboratory for preparation of slides. The slides with sections obtained were scanned through Trinocular Carl Zeiss's microscope (Germany) under different magnifications. Changes if any in the cyto-architecture were noted down.

Procedures followed to prepare histopathological slides

Fixation: The tissues were excised out immediately after sacrificing the animals, cleaned of extraneous tissues, cut into pieces of appropriate thickness and were transferred to 10% formalin solution. The tissues were allowed to remain in it till they are taken up for processing.

Tissue processing: The tissue processing involves dehydration, clearing and infiltration of the tissue with paraffin. The usual dehydrating agent is ethyl alcohol; acetone and isopropyl alcohol can also be used. Following dehydration tissue was transferred to a paraffin solvent, which is miscible with the dehydrating agent as well. These are known as clearing agent such as chloroform and xylene. The tissue were thoroughly washed by placing them under running tap water and then passed through a series of following solvents as per schedule for dehydration, clearing and paraffin infiltration.

Alcohol 70 % : 20 min.
Alcohol 80% : 20 min.
Alcohol 90% : 20 min.
Alcohol 95 % : 20 min.
Isopropyl Alcohol : 20 min.
Acetone (2 changes) : 20 min.
Chloroform (3 changes) : 20 min.
Melted paraffin wax (60^0c), (2 changes): 30 min. each

Next the tissues were embedded in paraffin wax to prepare tissue blocks, which are oriented so that sections are cut in desired plane of the tissue. Tissue blocks were fixed to a metal object holder after trimming them to suitable size.

Section cutting

The tissue section of the 5-6μm thickness were cut with the help of Spencer type rotating microtone and floated in a water bath between 50-55^0c for 30 minutes and then they were mounted on clear glass slides with a drop of Mayer's egg albumin dried on hot plate at 50^0c for 30 minutes.

Staining

After fixing the section on the slide, the sections were stained by serially placing them in the following reagents.

Xylol (2 changes) : 3 min.
Acetone : 3 min.
Alcohol 95 % : 3 min.
Running water : 3 min.
Haematoxylin stain : 20 min.
Running water wash : 20 min.
Eosin working solution : 2 min.
Alcohol 95% (3 changes) : 3 min.
Acetone (2 changes) : 3 min.
Xylol (2changes) : 3 min.

After passing through all the above reagents and stains, the slides were covered with D.P.X (Diphenyl Pthaliene Xylene) and cover slip were placed. Care was taken to avoid the air bubble formation during mounting the slide.

The sliders were viewed under binocular research Carl-Zeiss's microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Statistical Analysis: All data were expressed as Mean±S.D. The statistical analysis was done by One way ANOVA (Tukey) followed by Dunnett`s Multiple Comparison `t` test as post- HOC test using Graph pad InStat version 3.00 for Windows 98, Graph pad Software, San Diego, California, USA. P-value considered as significant are indicated by "*" and "**" for $p<0.05$ and $p<0.01$ respectively.

The data related to the effect of Gandhaprasaarini on Serum Urea level after 3 hour of PO injection have been depicted in Table 1. The data shows there was increase in serum urea level in PO control group when compared to the normal control group, the observed increase was found to be statistically non significant. The data shows there was increase in serum urea level in Standard group when compared to the PO control group, the observed increase was found to be statistically non significant. The data shows there was decrease in serum urea level in T1 group when compared to the PO control group, the observed decrease was found to be statistically non significant. The data shows there was decrease in serum urea level in T2 group when compared to the PO control group, the observed decrease was found to be statistically significant.

The data related to the effect of Gandhaprasaarini on Serum Urea level after 24 hours of PO injection have been depicted in Table 2. The data shows there was decrease in serum urea level in PO control group when compared to the normal control group, the observed decrease was found to be statistically non significant. The data shows there was increase in serum urea level in Standard group when compared to the PO control group, the observed increase was found to be statistically very significant. The data shows there was increase in serum urea level in T1group when compared to the PO control group, the observed increase was found to be statistically non significant. The data shows there was decrease in serum urea level in T2group when compared to the PO control group, the observed decrease was found to be statistically non significant.

The data related to the effect of Gandhaprasaarini on Serum Uric Acid level after 3 hours of PO injection have been depicted in Table 3. The data shows there was increase in serum uric acid level in PO control group when compared to the Normal control group, the observed increase was found to be statistically very significant. The data shows there was decrease in serum uric acid level in Standard group when compared to the PO control group, the observed decrease was found to be statistically very significant. The data shows there was increase in serum uric acid level in T1 and T2 groups when compared to the PO control group, the observed increase was found to be statistically non significant.

The data related to the effect of Gandhaprasaarini on Serum Uric Acid level after 24 hours of PO injection have been depicted in Table 4. The data shows there was increase in serum uric acid level in PO control group when compared to the Normal control group, the observed increase was found to be statistically non significant. The data shows there was decrease in serum uric acid level in Standard group when compared to the PO control group, the observed decrease was found to be statistically non significant. The data shows there was increase in serum uric acid level in T1 group when compared to the

PO control group, the observed increase was found to be statistically non significant. The data shows there was decrease in serum uric acid level in T2 group when compared to the PO control group, the observed decrease was found to be statistically non significant.

The data related to the effect of Gandhaprasaarini on Serum Creatinine level after 3 hours of PO injection have been depicted in Table 5. The data shows there was increase in serum creatinine level in PO control group when compared to the Normal control group, the observed increase was found to be statistically non significant. The data shows there was increase in serum creatinine level in Standard group when compared to the PO control group, the observed increase was found to be statistically non significant. The data shows there was increase in serum creatinine level in T1 group when compared to the PO control group, the observed increase was found to be statistically very significant. The data shows there was increase in serum creatinine level in T2 group when compared to the PO control group, the observed increase was found to be statistically non-significant.

The data related to the effect of Gandhaprasaarini on Serum Creatinine level after 24 hours of PO injection have been depicted in table 6: The data shows there was increase in serum creatinine level in PO control group when compared to the Normal control group, the observed increase was found to be statistically non significant. The data shows there was decrease in serum creatinine level in Standard group when compared to the PO control group, the observed decrease was found to be statistically non significant. The data shows there was increase in serum creatinine level in T1 andT2 groups when compared to the PO control group, the observed increase was found to be statistically non significant.

The data related to the effect of Gandhaprasaarini on kidney weight after 24 hours of PO injection have been depicted in Table 7. The data shows there was decrease in kidney weight in PO control group when compared to the Normal control group, the observed decrease was found to be statistically non significant. The data shows there was increase in kidney weight in Standard group when compared to the PO control group, the observed increase was found to be statistically significant. The data shows there was increase in kidney weight in T1 and T2 groups when compared to the PO control group, the observed increase was found to be statistically non significant.

The data related to the effect of Gandhaprasaarini on Body weight after 24 hours of PO injection have been depicted in Table 8. The data shows there was decrease in body weight gain in PO control group when compared to the Normal control group, the observed decrease was found to be statistically non significant. The data shows there was decrease in body weight gain in Standard and T1 groups when compared to the PO control group, the observed decrease was found to be statistically non significant and Gain in T2 group when compared to the PO control group, the observed increase was found to be statistically non-significant.

The data related to the effect of Gandhaprasaarini on Uric Acid Crystal level after 24 hours of PO injection have been depicted in Table 9. The data shows there was decrease in uric acid crystal level in Standard group when compared to the PO control group, the observed decrease was found to be statistically non significant. The data shows there was increase in uric acid crystal level in T1 and T2 groups when compared to the PO control group, the observed increase was found to be statistically very significant.

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Table 1: Effect of Gandhaprasaarini on Serum Urea – After 3hours

Group	Urea (mg/dL)	% Change
Normal Control	36.8±1.31	-
Potassium Oxonate Control	40.42±3.86	9.84↑@
Standard (Allopurinol)	56.0±12.43	38.54↑#
Gandhaprasaarini T1 (TED)	40.16±6.54	0.64↓#
Gandhaprasaarini T2 (2×TED)	16.83±0.87*	58.36↓#

Data: MEAN ±SEM * P<0.05, @-compared with Normal control, #-compared with PO control

Table 2: Effect of Gandhaprasaarini on Serum Urea – After 24hours

Group	Urea (mg/dL)	% Change
Normal Control	36.8±1.31	-
Potassium Oxonate Control	31.57±3.99	14.21↓@
Standard (Allopurinol)	64.86±14.82**	105.45↑#
Gandhaprasaarini T1 (TED)	38.66±4.90	22.49↑#
Gandhaprasaarini T2 (2×TED)	27.83±1.64	11.85↓#

Data: MEAN ±SEM, **P<0.01, @-compared with Normal control, #-compared with PO control

Table 3: Effect of Gandhaprasaarini on Serum Uric Acid – After 3hours

Group	Uric acid (mg/dL)	% Change
Normal Control	1.08±0.09	-
Potassium Oxonate Control	2.50±0.41**	131.48↑@
Standard (Allopurinol)	0.71±0.21**	71.6↓#
Gandhaprasaarini T1 (TED)	2.66±0.17	6.4↑#
Gandhaprasaarini T2 (2×TED)	3.10±0.04	24↑#

Data: MEAN ±SEM, **P<0.01,@-compared with Normal control, #-compared with PO control

Table 4: Effect of Gandhaprasaarini on Serum Uric Acid – After 24hours

Group	Uric acid (mg/dL)	% Change
Normal Control	1.08±0.09	-
Potassium Oxonate Control	1.46±0.15	35.18↑@
Standard (Allopurinol)	1.04±0.24	28.77↓#
Gandhaprasaarini T1 (TED)	1.80±0.11	23.29↑#
Gandhaprasaarini T2 (2×TED)	1.41±0.01	3.42↓#

Data: MEAN ±SEM, @-compared with Normal control, #-compared with PO control

Table 5: Effect of Gandhaprasaarini on Serum Creatinine – After 3hours

Group	Creatinine (mg/dL)	% Change
Normal Control	0.26±0.04	-
Potassium Oxonate Control	0.37±0.07	42.31↑@
Standard (Allopurinol)	0.38±0.10	2.70↑#
Gandhaprasaarini T1 (TED)	0.76±0.05**	105.40↑#
Gandhaprasaarini T2 (2×TED)	0.42±0.06	13.51↑#

Data: MEAN ±SEM, **P<0.01, @-compared with Normal control, #-compared with PO control

Table 6: Effect of Gandhaprasaarinion Serum Creatinine – After 24hrs

Group	Creatinine (mg/dL)	% Change
Normal Control	0.26±0.04	-
Potassium Oxonate Control	0.37±0.07	42.31↑@
Standard (Allopurinol)	0.23±0.09	37.84↓#
Gandhaprasaarini T1 (TED)	0.46±0.06	24.32↑#
Gandhaprasaarini T2 (2×TED)	0.45±0.05	21.62↑#

Data: MEAN ±SEM, @-compared with Normal control, #-compared with PO control

Table 7: Effect of Gandhaprasaarini on Kidney Weight

Group	Kidney weight (g)	% Change
Normal Control	1.46±0.08	-
Potassium Oxonate Control	1.26±0.04	13.70↓@
Standard (Allopurinol)	1.61±0.15*	27.78↑#
Gandhaprasaarini T1 (TED)	1.54±0.02	22.22↑#
Gandhaprasaarini T2 (2×TED)	1.29±0.03	2.38↑#

Data: MEAN ±SEM,*P<0.05, @-compared with Normal control, #-compared with PO control

Table 8: Effect of Gandhaprasaarini on % change in Body Weight gain

Group	Body weight (g)	% Change
Normal Control	7.74±1.33	-
Potassium Oxonate Control	3.30±1.81	57.36↓@
Standard (Allopurinol)	0.31±1.95	90.61↓#
Gandhaprasaarini T1 (TED)	2.48±6.49	24.85↓#
Gandhaprasaarini T2 (2×TED)	5.58±3.86	69.09↑#

Data: MEAN ±SEM, @-compared with Normal control, #-compared with PO control

Table 9: Effect of Gandhaprasaarini on Uric Acid Crystal in Urine

Group	Uric acid crystal in urine (crystals/cu.mm of urine deposit)	% Change
Potassium Oxonate Control	5640.0±3064.0	-
Standard (Allopurinol)	3583.33±118.79	36.46↓
Gandhaprasaarini T1 (TED)	21706.66±1432.1**	284.87↑
Gandhaprasaarini T2 (2×TED)	16533.33±2242.5**	193.14↑

Data: MEAN ±SEM, **P<0.01

Histological changes

Control Group

Microscopic examination of the kidney sections from potassium oxonate control group revealed tubular dilation, cell infiltration, proteus changes and few crystals in the tubules. Representative photomicrographs from Control group can be found in Figures 2a to 2f.

Standard Group

In Allupurinol administered group these changes were found to be comparatively less; however, mild to moderate cell infiltration was observed.

Representative photomicrographs from Standard group can be found in Figures 3a to 3f.

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Urine Microscopy

Figure 1a

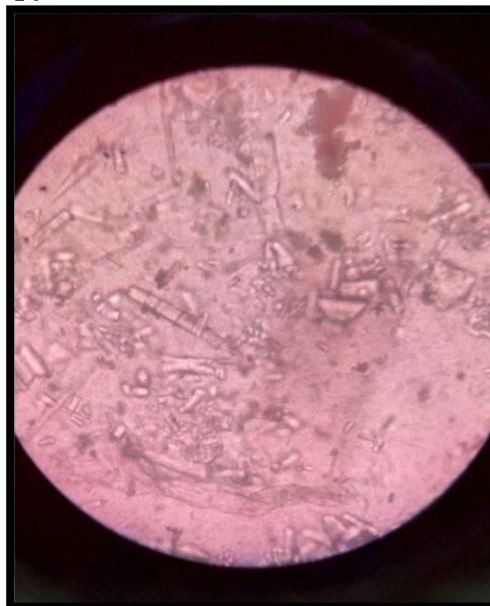

Figure 1b

TED Group

In TED dose Gandhaprasaarini administered group crystals were very few, cell infiltration was mild at only one or two sites, proteus changes were mild seen only in two rats, tubular dilatation was much less. The overall intensity was much less in comparison to PNO_3 control group. Representative photomicrographs from TED Group can be found in Figures 4a to 4f.

TED x 2 Group

In TED x 2 dose Gandhaprasaarini administered group also good protection was observed – cell infiltration, tubular dilatations were mild and not seen in all the rats. Proteus changes were not seen in any of the sections. Fatty changes and necrosis were also not observed.

The overall protective effect was the best among all the groups tested. Representative photomicrographs from TEDx 2 Group can be found in Figures 5a to 5f.

DISCUSSION

Effecton Serum Uric Acid

Analysis of the data shows that there is significant increase in serum uric acid in positive control at 3rd hour after injection. It is due to- administration of potassium oxonate, an inhibitor of urate oxidase. Further it has to be observed that, there is significant decrease in standard group due to standard drug Allopurinol and its active metabolite, oxypurinol, which inhibits the enzyme xanthine oxidase, blocking the conversion of the oxypurines, hypoxanthine and xanthine to uric acid. Elevated concentrations of oxypurine and inhibition of xanthine oxidase through negative feedback results in a decrease in the concentrations of uric acid in the serum. In the test drug administered group no such decrease was observed, instead a mild increase was observed. This may be indicative that the test drug may not share the mechanism of action seen in allopurinol.

The data pertaining to 24 h sample show that the acute elevation observed at 3rd hour was not sustained. The reference standard showed non-significant decrease, similar effect was observed with TED x2 dose of the test drug and the TED dose produced non-significant increase.

Effect on Serum Urea

In positive control a moderate elevation in serum urea was observed. This may be indicative of higher turnover of nitrogenous substances. In Allupurinol group there was non-significant further increase- this may be due to the decrease in the metabolism of uric acid. In test drug administered group a non-significant decrease was observed. TED x2 dose of the test drug showed a significant decrease in serum urea level. This parameter was measured to ascertain whether the observed hyperuricaemic condition significantly affects kidney function or not. Though histopathological changes were observed in the positive control group- it does not seems to be sufficient to markedly affect kidney function. This further evident in the fact after 24 h a mild decrease was observed in positive control group.

The data pertaining to 24 h sample show that the acute elevation observed at 3rd hour was not sustained. The reference standard showed highly significant increase, whereas non-significant decrease was observed with TED x2 dose of the test drug and the TED dose produced non-significant increase.

Effect of on Serum Creatinine

Serum creatinine is an important indicator of renal health because creatinine is a non-protein waste product of creatinine phosphate metabolism by skeletal muscle tissue. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidney become impaired for any reason, the creatinine level in the blood will raise due to poor clearance of creatinine by the kidneys.

In the present study the data obtained shows a non-significant decrease in serum creatinine in positive control group at 3rd hour and a non-significant decrease at 24 hour. This again show effect similar to that one observed in case of serum urea level. In reference standard and higher dose test (TED x 2 doses) groups no significant effect could be observed in 3rd hour sample- only mild changes were observed. In TED dose given group significant elevation was observed at 3rd hour and the increase was non-significant at 24 h.

This is not indicative of functional impairment in the kidney- since this effect was not sustained up to 24 h and histological examination showed good reversal of the toxicant induced changes. Thus it may be transient elevation due to increased turnover which is self limiting in nature.

The data related to the above parameters indicate that though hyperuricemic conditions was induced by administration of potassium oxonate in the used dose , the observed effect is not sufficient to cause significant elevation in the above parameters. However, changes were observed in microscopic profile of the kidney, indicating the protocol employed is good for predicting effect of test drug on hyperuricemic condition.

Effect on Kidney weight

When compared with Positive control group; Standard control group, Test group1 and 2 showed increase in kidney weight. As could be observed administration of the toxicant lead to non-significant decrease in kidney weight.

Hence no inference could be made. However, it can be suggested that the apparent non-significant increase observed may be indicative of reversal of the toxicant’s effect.

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Histopathology of Kidney (Control group)

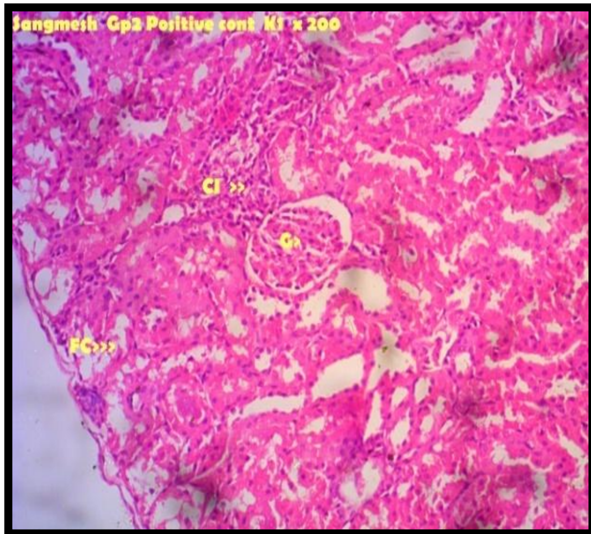

Figure 2a

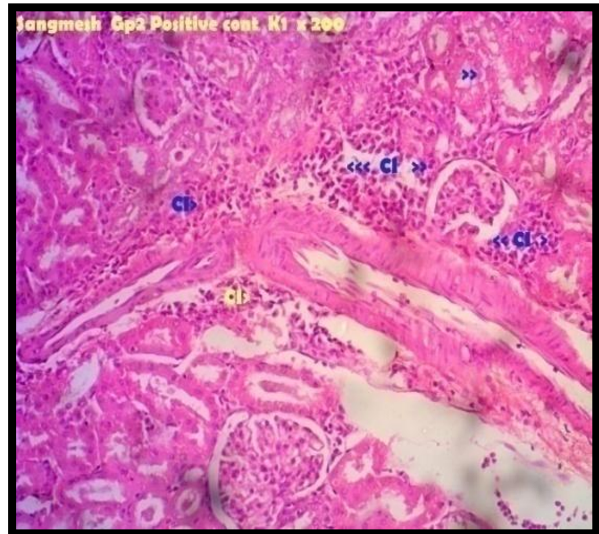

Figure 2b

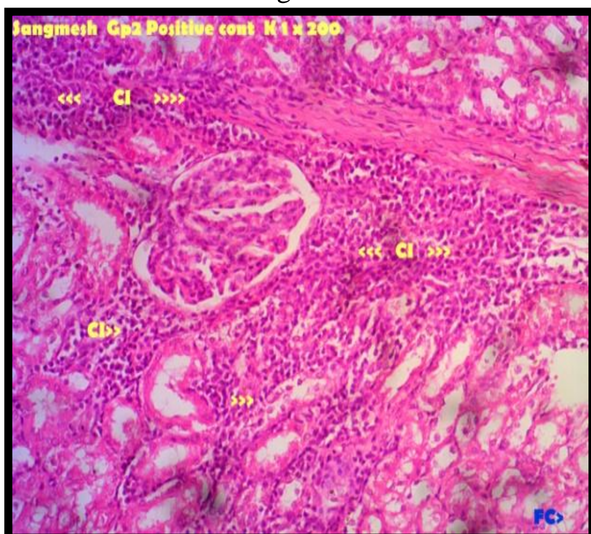

Figure 2c

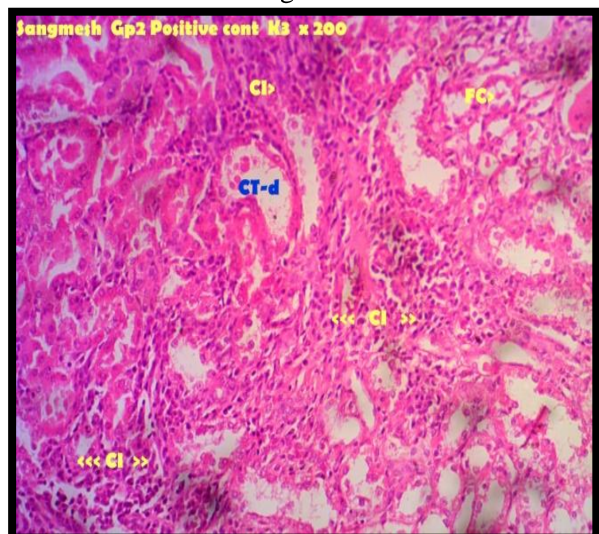

Figure 2d

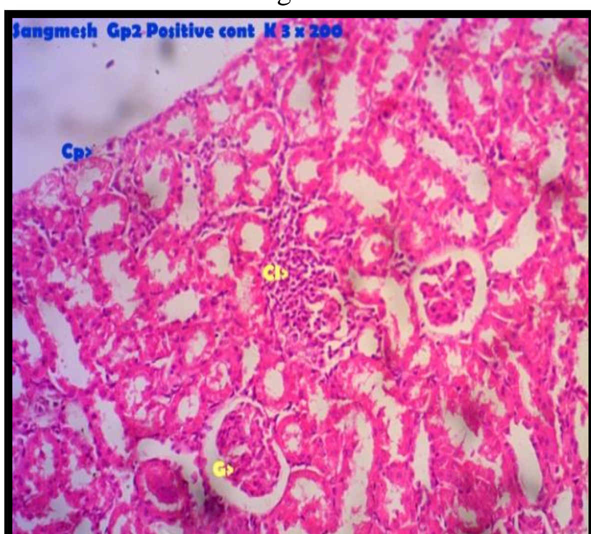

Figure 2e

Figure 2f

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Histopathology of Kidney (Standard group)

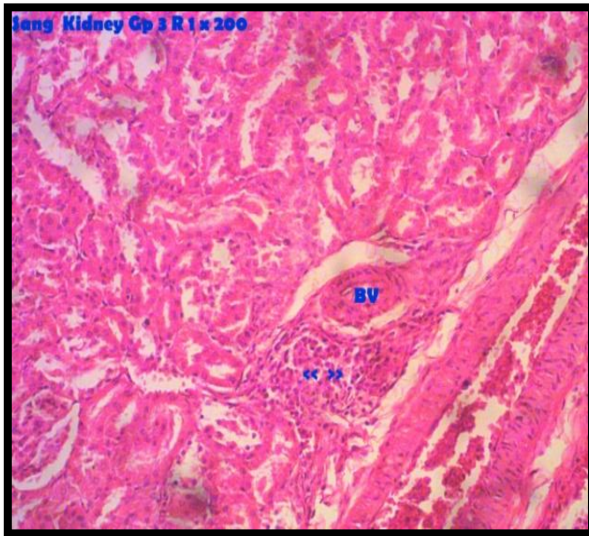


Figure 3a

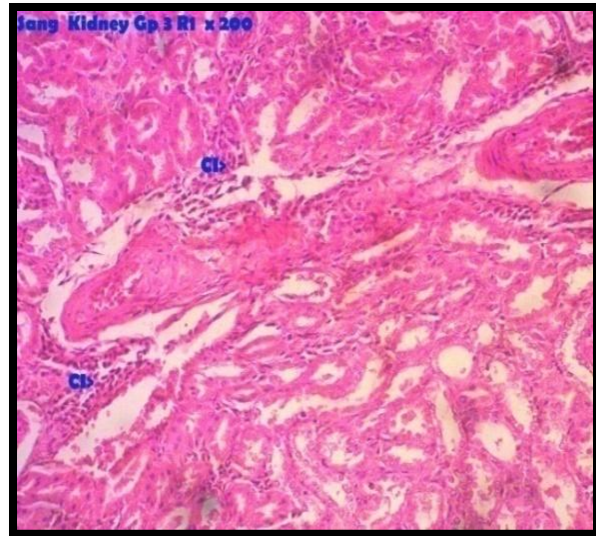
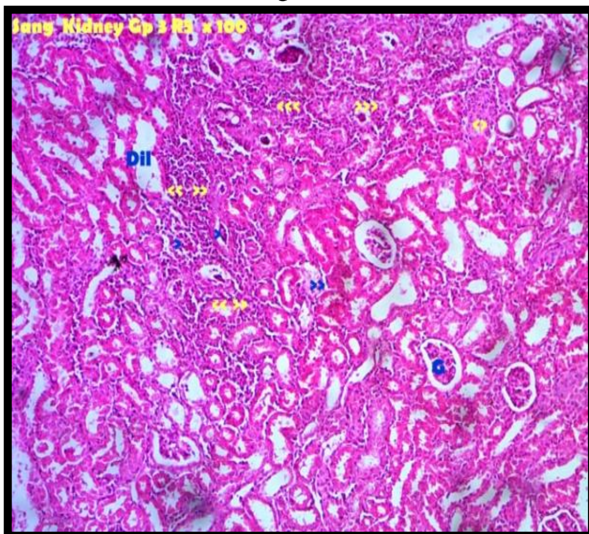


Figure 3b



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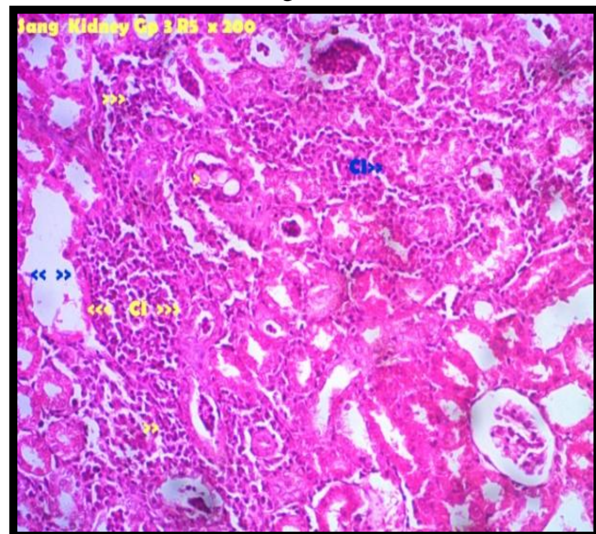

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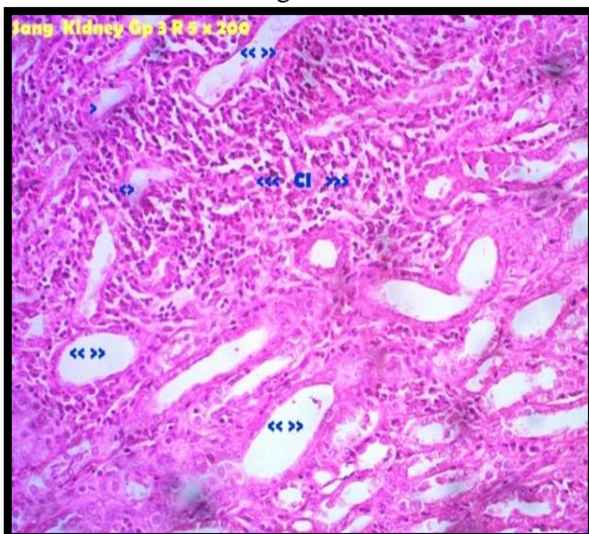

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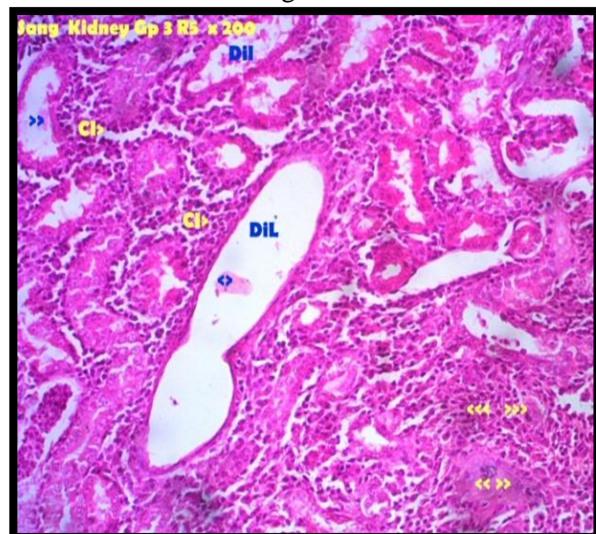


Figure 3f

Histopathology of Kidney (Trial drug - T1 group)

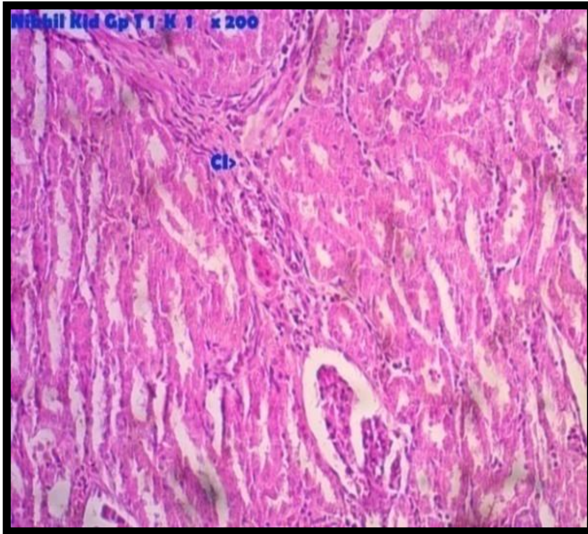


Figure 4a

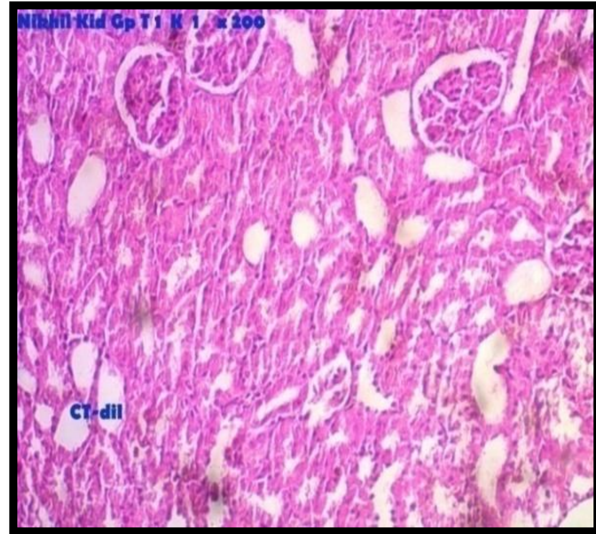
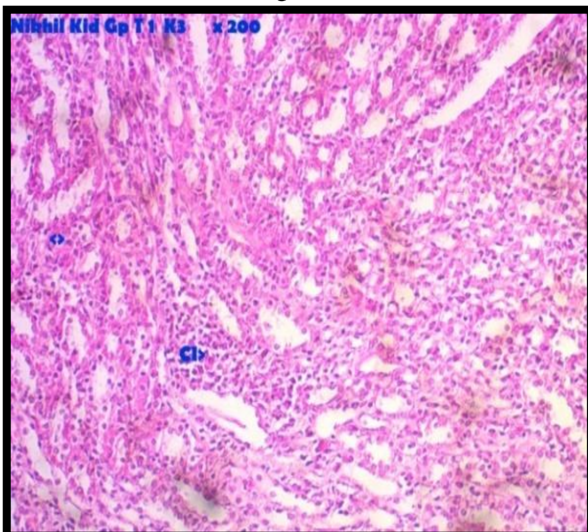


Figure 4b



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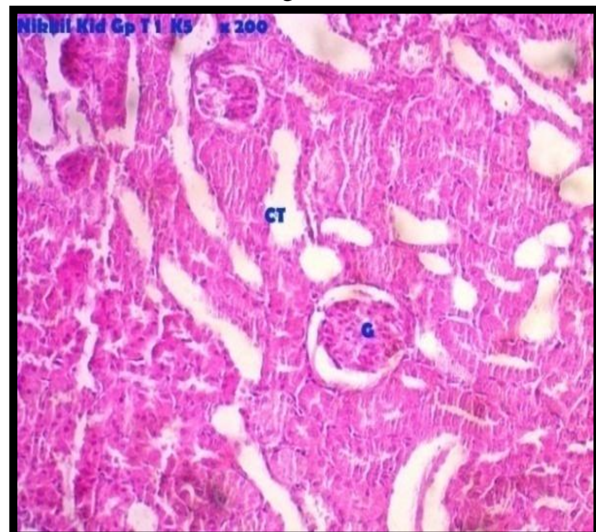

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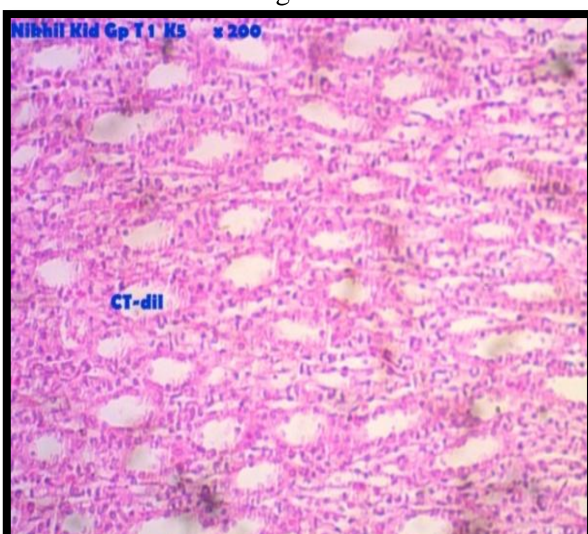

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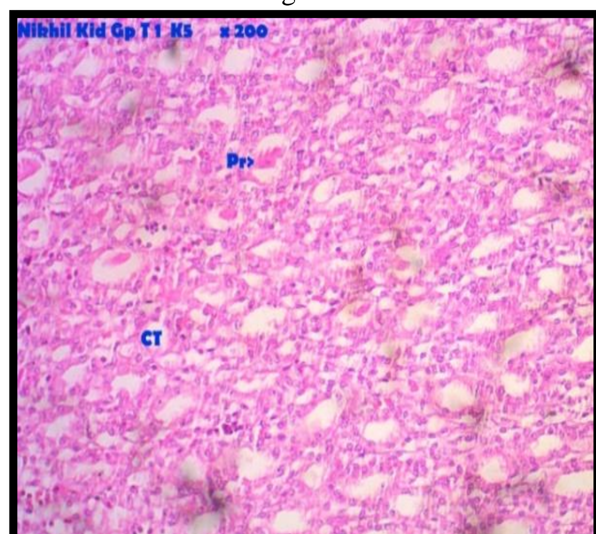

Figure 4f

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Histopathology of Kidney (Trial drug – T2 group)

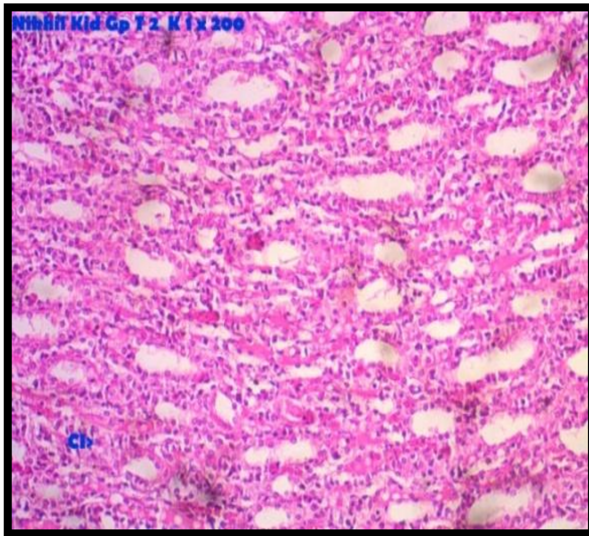


Figure 5a

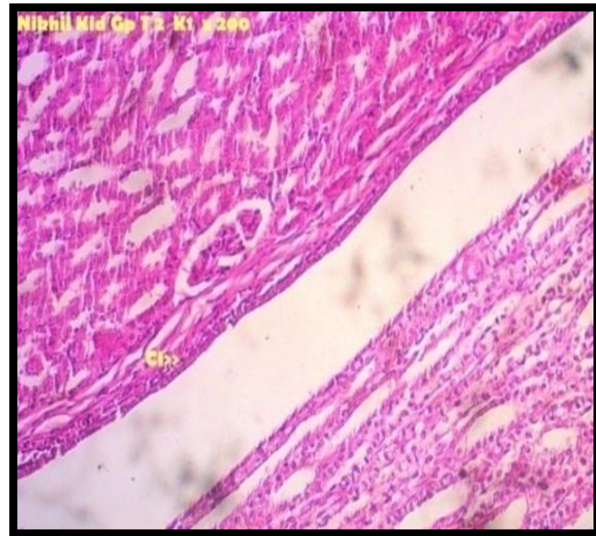


Figure 5b

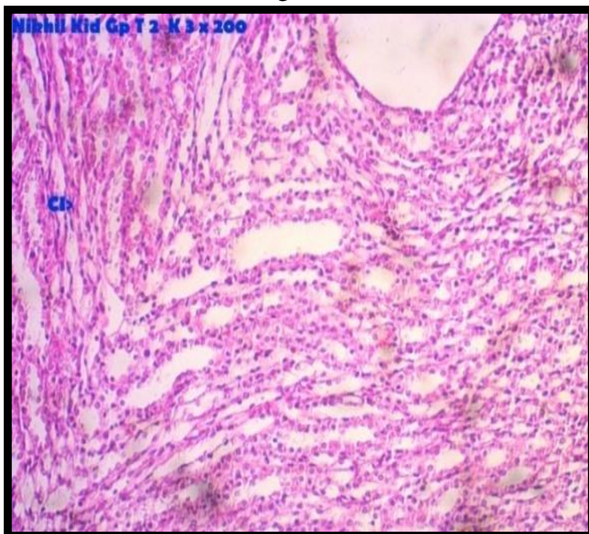


Figure 5c

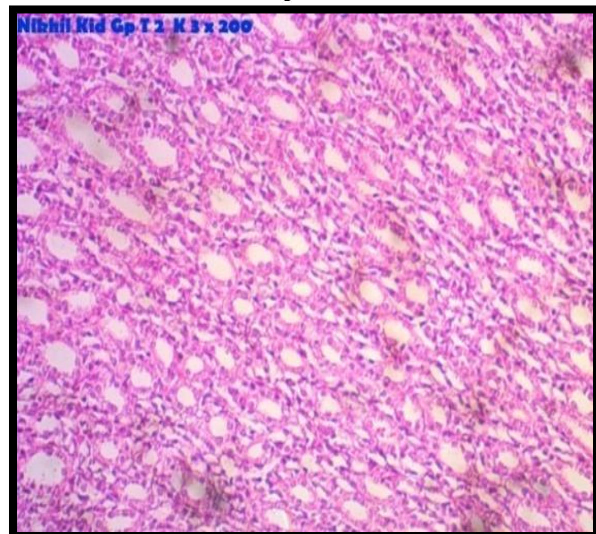
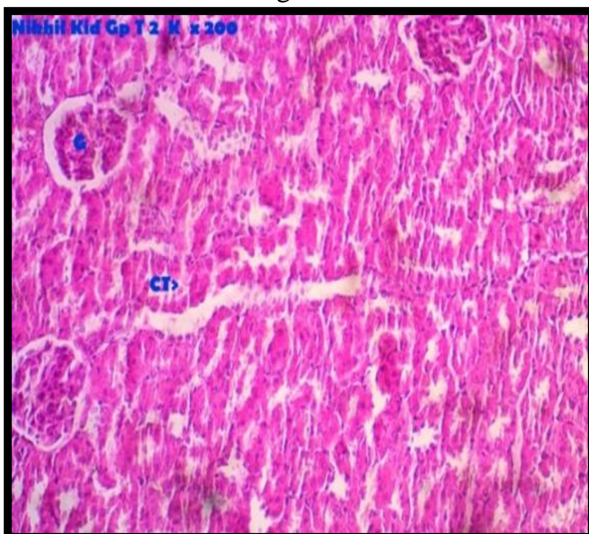


Figure 5d



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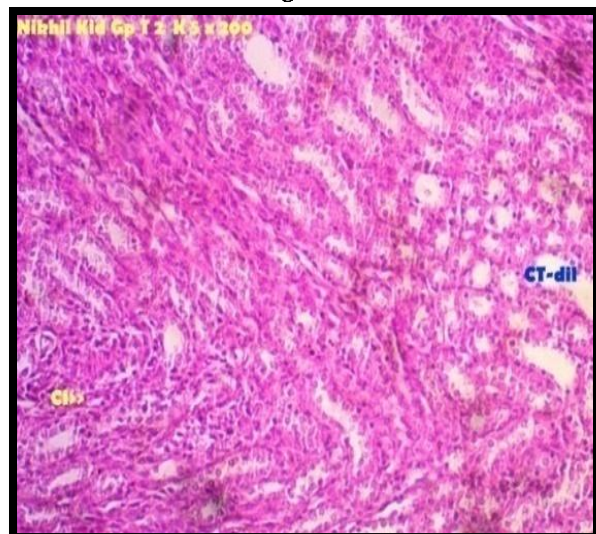

Figure 5f

Histopathology Markers
Positive Control Group: CI = Cell Infiltration; G = Glomerulus; FC = Fatty Changes; CT-d = Convoluted Tubule dilated; Cp = Capsule.
Standard Group: CI = Cell Infiltration; BV = Blood Vessel; G = Glomerulus.
T1 Group: CI = Cell Infiltration; G = Glomerulus; CT-d = Convoluted Tubule dilated Pr = Proteus changes
T2 Group: Pr = Proteus changes; CI = Cell Infiltration; G = Glomerulus; CT-d = Convoluted Tubule dilated

Effect on % change in Body weight gain

When compared with Positive control group; Standard control group showed apparent non significant reduction in body weight gain. Test group 1 showed slight non-significant decrease in body weight gain whereas body weight gain increased non-significantly in Test group2. Test drug Gandhaprasaarini doesn’t affect the normal metabolism when compared with Positive control and Standard group.

Effect on Uric Acid Crystal

Administration of potassium oxonate leads to observation of uric acid crystals in the urine. The uric acid crystal content was found to be remarkably increased in both the test drug administered group. This increased urinary excretion of the uric acid crystals may be the reason for the protective effect observed with test formulation as observed in histological examination.

Histopathological Study

Histopathological examination of the kidney sections showed moderate degenerative changes in the potassium oxonate administered group. These changes were moderately reversed in Allupurinol administered and potassium oxonate injected group. The effect was much better in both the test drug administered group. This indicates that the test formulation has better kidney protective effect. Analysis of the result obtained in this study indicates that the experimental protocol employed in this study is effective in inducing hyperuricaemic condition. The test drug did not significantly reverse the observed acute elevation in the serum uric acid level but observed a significant decrease in serum urea level immediately after inducing hyperuricaemia. However, it produced significant increase in the excretion of uric acid crystals in the urine. This indicates presence of significant uricosuric effect. This may be the reason for the observed remarkable effect against the toxicant induced degenerative changes observed microscopically. XO is the key enzyme in the catabolism of purines and has a critical role in the endogenous production of uric acid. This study provides evidence for the use of this formulation with xanthine oxidase inhibitors clinically. The efficacy of such a combination can be assessed in future research efforts.

Possible Mode of action of the Drug

Gandhaprasaarini has Tikta rasa, guru-saraguna, Ushnavirya, and katuvipaka. It has been mentioned in the classics that certain drugs act through Rasa, some through Virya, some through their Gunas, some through their Vipaka and some through their Prabhava.[13] It is further mentioned that Guna is more powerful than Rasa and Virya is still more powerful than Guna.[14] Tikta rasa alleviates rakta and pitta doshas, so it act as anti inflammatory. Sara guna of the drug Gandhaprasaarini suggests the uricosuric action which helps in reducing uric acid level by excreting through urine. Ushna Virya classically acts against both Vata and Kaphadosha which is commonly seen in inflammatory conditions. Presence of coumarins in the drug indicates the anti inflammatory action of the drug. Leaves contain Vitamin C which is a vital anti oxidant that helps to protect cell from damage caused by free radicals. In a randomized controlled trial it was found that Vit. C flushes out uric

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acid through urine and thereby reducing serum uric acid level and also it has showed nephroprotective action. Leaves are also high in Calcium and rich in Potassium. Various minerals analysis study showed presence of Ca, Na, Fe which are essential for human beings. Fe is a trace element essential to make haemoglobin and myoglobin, and a number of enzymes peroxidase, catalase, hydroxylase, and flavin enzymes. Previous studies shows the dual actions of *Paederia scandens* extract as a hypouricemic agent: xanthine oxidase (XO) inhibitory activity and uricosuric effect.

CONCLUSION

Analysis of the result obtained in this study indicates that the experimental protocol employed in this study is effective in inducing hyperuricaemic condition. The test drug showed a significant decrease in serum urea level immediately after inducing hyperuricaemia. It produced significant increase in the excretion of uric acid crystals in the urine which indicates presence of significant uricosuric effect. This may be the reason for the observed remarkable effect against the toxicant induced degenerative changes observed microscopically. Thus it provides evidence for the use of this formulation with xanthine oxidase inhibitors clinically. Histopathological examination of the kidney sections showed moderate degenerative changes in the potassium oxonate administered group (Positive control). These changes were moderately reversed in allupurinol administered (Standard) and potassium oxonate injected group. The effect was much better in both the test drug administered group. This indicates that the test formulation has better kidney protective effect.

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Source of Support: Nil Conflict of Interest: None Declared