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Original Article
In vivo studies

Pharmacological Evaluation of Bark of *Ficus religiosa* Linn. in Vata Rakta with Special Reference to Hyperuricemia

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ABSTRACT

Introduction: *Ficus religiosa* Linn. (Moraceae) is a pharmacopoeial drug of Ayurveda, commonly known as peepal in Hindi. This is a source drug for the plant *Ashvattha* in Ayurveda. The plant has a religious and ancient history found all over India. The tree is found to be sacred because of its religious rituals. It has also got the importance in medicinal science and hence used in different systems of medicine such as Ayurveda, Unani and Homeopathy. The drug is considered to be precious because of its wide therapeutic efficacy. The different parts of this plant like bark, leaves, tender shoots, fruit, seed, latex etc. are being used for different ailments since ages. Acharyas in samhita have mentioned the use of *Ashvattha* in various contexts. *Vata rakta* is one such disease where the bark *kashaya* of *Ashvattha* is used. One of the correlations for *vata rakta* in modern science is gout in which one of the parameters assessed is hyperurecemia. Here the pharmacological study is conducted as a preclinical study by using different experimental models. ***Methods:*** The experimental study was carried out in the Pharmacology department of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The anti-hyperuricemic activity has been assessed by Potassium oxonate induced hyperuricemic model. ***Result:*** The experimental study had shown bark *kashaya* as extremely significant values in serum uric acid and urine uric acid parameters in anti-hyperuricemic activity. ***Conclusion:*** The experiment has shown *Ashvattha* bark to have potential anti-hyperuricemic activity.

KEYWORDS

Bark, Kashaya, Medicinal use, Pharmacological study, Pharmacopeial drug.

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INTRODUCTION

The tree, *Ashvattha* (*Ficus religiosa* Linn. - Moraceae) is also known as sacred tree or sacred fig, is an important drug which is being used in Ayurveda since ages. The mentioning of the drug *Ashvattha* can be seen in classical Ayurveda Samhita as well as in Nighantus.[1-2] In India, since ancient times it has got great mythological, religious and medical importance. This plant is considered as oldest plant in Indian art literature. The mentioning of Ashvattha can be seen in Vedas, Puranas, Upanishads, Bhagavat Gita etc. According to religious point of view, *F. religiosa* has got importance as it is believed that Gauthama Buddha got enlightened under this tree and hence the plant is called as 'Bodhee tree' or 'Bo tree'. It is said in many contexts in various samhitas. Out of which Acharya charaka have mentioned it in vata shonita adhyaya. Acharya clearly mentions about the use of *Ashvattha twak kashaya* along with the adjuvant honey as best remedy for *vata rakta*.[3] *Vata rakta* which can be one of the correlations as gout is assessed by the parameter hyperuricemia (Increased uric acid level in blood). Animal experimental study are said to be the easiest and safest method for conducting the experiments. The demand for single drug therapy is gaining its importance in global level. Here an attempt is made to evaluate the action of *Ficus religiosa* bark kashaya which is a single drug therapy for *vata rakta* in Hyperuricemia in potassium oxonate induced hyperurecemic animal model.

MATERIALS AND METHODS

Drug preparation

For single dose *kashaya*, dried sample of *Ashvattha* was taken and it is pounded in *khalva yantra* to obtain course powder. Then 12 g of prepared *kwatha churna* is weighed and taken, in that 16 parts of water is added ie, 192 ml of water is added and reduced to 1/8th. i.e; the *kashaya* obtained was 24 ml for administering kashaya to 6 rats by calculating the animal dose according to the body weight.

For double dose *kashaya*, 24 g of prepared *kwatha churna* is weighed and taken, 384 ml of water is added to the *kwatha churna* and it is reduced to 1/8th. i.e; the *kashaya* will be 48 ml. Since the rats can't be given so much of kashaya in volume so in order to avoid the practical difficulty the concentration of the kashaya is increased by reducing it to 25 ml.

Experimental Animal

Wistar albino rats of either sex, weighing 200 ± 50 g was selected for the study. The animals were obtained from Animal house attached to the Pharmacology laboratory of S. D. M Research Centre Udupi. Rats were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to experimentation. The

experimental rats were maintained at 12:12h light and dark cycle. Temperature of 25^{0}C and relative humidity of approximately 50%. The experimental protocol was approved by the institutional animal ethical committee (558/02/C/CPCSEA/SDMCRA/IAEC/DG 03/2016-17).

Dose selection

The dose of *Ashvattha kashaya* for human use is 2 *pala* (96 ml) according to classical text of *Bhaishajya kalpana*.[4] The rat dose was calculated from adult human dose based on body surface area ratio by referring to the Paget and Barn's table 1964. The calculated dose was found to be 9 ml/kg body weight and considered as therapeutic dose (TED).[5] The *Ashvattha kashaya* was administered in two different doses range i.e. therapeutic (TED) and two times therapeutic dose 18 ml/kg (TED X 2) respectively. Since the drug is given only for once a day, the single and double dose will give a clear idea to compare that in which dose it will have maximum potency. The positive control and standard drug was made suspension in 0.5 % CMC (Carboxy methyl cellulose) and administered at a dose of 1.5 ml/100g body weight with the help of oral catheter.

Study design

30 Wistar albino rats of either sex weighing 200 ± 50 g were selected and grouped five. Then the body weight is taken. There after rats were stained on head, neck, body, tail, forelimb and one with no marks for identification purpose and the dose calculation was made by considering the body weight of each rat. Animals of Group-I was administered with normal water and diet for 7 consecutive days and served as normal control. Group II administered with 0.5% CMC orally and served as positive control. Group III administered with Allopurinol 180mg/kg (Human dose) orally for 7 consecutive days and served as reference standard. Group IV & V administered with test drug at therapeutic and double the therapeutic dose for 7 consecutive days and served as trial group. On 7th day for group 2, 3, 4 and 5 after an hour of administration of group specific drug Potassium Oxonate 300 mg/kg is given intra peritonially and rats will be anaesthetized to collect blood from retro-orbital plexuses after 3 hours of drug administration for investigation. Serum uric acids, Blood urea, Serum Creatinine were evaluated to see the trial drug significance. Then the rats were kept in a metabolic cage for collecting the urine sample. Urine sample is collected after 24 hours of introducing to metabolic cage for Urine uric acid investigation. Then the rats are re-anesthetized and blood should be drawn from retro orbital plexus, after which the rats of each group are selected alternatively for organ histopathological study and sacrificed (Fig. 1).[5-7]

The kidney of 3 rats which is selected alternatively from each group was examined for histopathalogical study. Immediately after the excision from animals, the kidneys were transferred into 10% formalin. Sections of 5 µm thickness of tissues were prepared using microtome and stained with haematoxyline and eosin for microscopic observations. All slides were then evaluated under light microscope (ZEISS Axio lab A1 India). [8]

Statistical analysis

The data obtained were presented as Mean ± SEM. The difference between or among the groups was analyzed by employing one-way ANOVA followed by Dunnet's multiple t-test as post hoc test. Graph pad Inst 3 was used for this purpose. A P value of less than 0.05 was considered to indicate statistically significant.[10]

RESULTS AND DISCUSSION

Effect of *Ashvattha kashaya* on serum uric acid measured after 3 hrs

Considering the uric acid level after 3 hours of drug induction, there was increase in the level of positive control compared to normal control which was statistically significant and there was decreased level of test I and test II when compared to positive control in which the difference was statistically non-significant (Table 1).

Table 1. Effect on Serum Uric acid measured 3 hrs after administration of Potassium oxonate

Group	Uric acid (mg/100 ml)	% change
Normal control	1.34 ± 0.07	--
Positive control	2.902 ± 0.22$^{@@}$	116.56 ↑ $^{@@}$
Reference standard (Allopurinol)	0.71 ± .0215 $^{\#\#}$	75.51 ↓$^{\#\#}$
Test I (*Ashvattha kashaya* – TED)	2.86 ± 0.33	1.37 ↓$^{\#\#}$
Test II (*Ashvattha kashaya* – TED × 2)	2.85 ± 0.342	1.72 ↓$^{\#\#}$

Data: MEAN ± SEM, ** P<0.01 $^{@@}$ - in comparison to normal control, $^{\#\#}$ - in comparison to positive control

Effect of *Ashvattha kashaya* on Serum Uric acid measured 24 hrs

In uric acid level after 24 hours there was decrease in standard and test I when compared to positive control where the data is **statistically very significant** and the decrease of the test II was noted as statistically non-significant. The reason for the changes in Serum Creatinine is not known. It is possible that Potassium oxonate affects its formation in the skeletal muscle there by reducing the load available to the kidney. Reversible of this decrease can be considered to represent the test drug induced reversal of toxicant's level effect. Thus it is an additional evidence to support the toxicant reversal effect of test drug (Table 2).

Effect of *Ashvattha kashaya* on Serum Creatinine measured after 3 hrs

In Serum Creatinine assessed after 3 hours of drug inducing, the test I show increase in the values which was statistically non-significant whereas test II shows increase in the values which was statistically very significant compared to the positive control. The positive control showed statistically significant decrease when compared to normal control and there was increase in standard group which is statistically non-significant when compared to positive control (Table 3).

Figure 1. Different procedures of experimental study

1.1 Bark collection

1.2 Collected bark

1.3 Kwatha churna

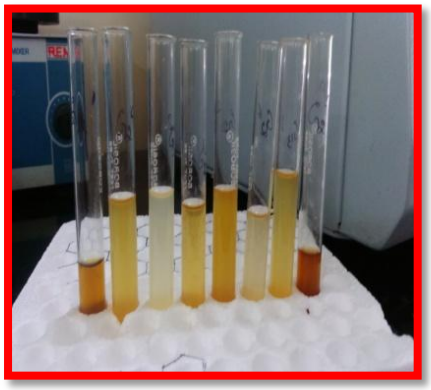
1.4 Preparation of kashaya

1.5 Kashaya

1.6 Feeding Kashaya

1.7 Inducing Potassium oxonate

1.8 Drawing blood



1.9 Urine sample

1.10 Dissection

1.11 Harvesting kidney

1.12 Kidneys for histopathology

Table 2. Effect on Serum Uric acid measured 24 hrs after administration of Potassium oxonate

Group	Uric acid (mg/100 ml)	% change
Normal control	1.294 ± 0.0593	---
Positive control	1.78 ± 0.222	37.56 ↑ @@
Reference standard (Allopurinol)	0.883 ± 0.216 ##	50.39 ↓ ##
Test I (*Ashvattha kashaya* – TED)	0.416 ± 0.030 ##	76.62 ↓ ##
Test II (*Ashvattha kashaya* – TED × 2)	1.55 ± 0.0500	12.92 ↓ ##

Data: MEAN ± SEM, **P<0.01 @@ in comparison to normal control, ## in comparison to positive control

Table 3. Effect on Serum Creatinine measured 3 hrs after administration of Potassium oxonate

Group	Serum Creatinine (mg/100 ml)	% change
Normal control	0.6 ± 0.036	---
Positive control	0.23 ± 0.033 @@	61.66 ↓ @@
Reference standard (Allopurinol)	0.32 ± 0.102	39.13 ↑ ##
Test I (*Ashvattha kashaya* – TED)	0.48 ± 0.066	108.69 ↑ ##
Test II (*Ashvattha kashaya* – TED × 2)	0.744 ± 0.092 ##	223.47 ↑ ##

Data: MEAN ± SEM, **P<0.01 @@ in comparison to normal control, ## in comparison to positive control

Effect of *Ashvattha kashaya* on Serum Creatinine measured after 24 hrs

Considering serum Creatinine after 24 hours, test I shows increase in data which was statistically very significant whereas test group II shows increase in data which was statistically non-significant when compared to positive control. There was decrease in the data of positive control and standard in which positive control is assessed to be statistically very significant when compared to the normal control and standard group shows statistically non-significant value when compared to positive control. Measurement of Blood urea did not provide any leads to determine the effect of toxicant on this parameter and impact of test drug administration on it. Hence not considered for drawing any inference (Table 4).

Table 4. Effect on Serum Creatinine measured 24 hrs after administration of Potassium oxonate

Group	Serum Creatinine (mg/100 ml)	% change
Normal control	0.6 ± 0.36	---
Positive control	0.25 ± 0.034 @@	58.33 ↓ @@
Reference standard (Allopurinol)	0.233 ± 0.095	6.8 ↓ ##
Test I (*Ashvattha kashaya* – TED)	1.483 ± 0.016 ##	493.2 ↑ ##
Test II (*Ashvattha kashaya* – TED × 2)	0.45 ± 0.07	80.0 ↑ ##

Data: MEAN ± SEM, ** P<0.01 @@ - in comparison to normal control, ## - in comparison to positive control

Effect of *Ashvattha kashaya* on Blood urea measured after 3 hrs

In blood urea assessed after 3 hours shows decrease in value of Test I and Test II and increase in Reference standard when compared to positive control. There was decrease in positive control when compared to normal control. All the said values are statistically non-significant (Table 5).

Table 5. Effect on Blood urea measured 3 hrs after administration of Potassium oxonate

Group	Blood urea (mg/100ml)	% change
Normal control	37.5 ± 1.76	---
Positive control	32.0 ± 2.302	14.66 ↓ @@
Reference standard (Allopurinol)	51.18 ± 10.73	59.93 ↑ ##
Test I (*Ashvattha kashaya* – TED)	28.0 ± 2.408	12.5 ↓ ##
Test II (*Ashvattha kashaya* – TED × 2)	26.8 ± 2.835	16.25 ↓ ##

Data: MEAN ± SEM, **P<0.01 @@ in comparison to normal control, ## in comparison to positive control

Table 6. Effect on Blood urea measured 24 hrs after administration of Potassium oxonate

Group	Blood urea (mg/100 ml)	% change
Normal control	37.5 ± 1.76	---
Positive control	25.16 ± 1.19	32.90 ↓ @@
Reference standard (Allopurinol)	64.86 ± 14.82 ##	157.79 ↑ ##
Test I (*Ashvattha kashaya* – TED)	28.4 ± 0.927	12.87 ↑ ##
Test II (*Ashvattha kashaya* – TED × 2)	27.8 ± 0.9695	10.49 ↑ ##

Data: MEAN ± SEM, ** P<0.01 @@ in comparison to normal control, ## in comparison to positive control

Effect of *Ashvattha kashaya* on Blood urea measured after 24 hrs

The blood urea assessed after 24 hours shows Increase in Test I and Test group II which was statistically non-significant when compared to positive control. There was increased value in Reference standard which can found statistically very significant when compared to positive control and the decreased value in positive control when compared to normal control was found to be statistically non-significant (Table 6).

Effect of *Ashvattha kashaya* on Urine uric acid

The urine uric acid shows decrease in standard and test I when compared to positive control but the data was found to be statistically non-significant. And the data was found to be increase in Test II where data is **statistically extremely significant.** Increased level in Urine may represent uricosuric effect – a desirable trait in the treatment of conditions like gout. Since it has both uric acid lowering in the serum and excretion in urine – it may be an ideal combination of two types of mechanism of action. However, exact correlation of these activities with the dose level was not observed. This requires further evaluation (Table 7).

Table 7. Effect on Urine uric acid measured after administration of Potassium oxonate

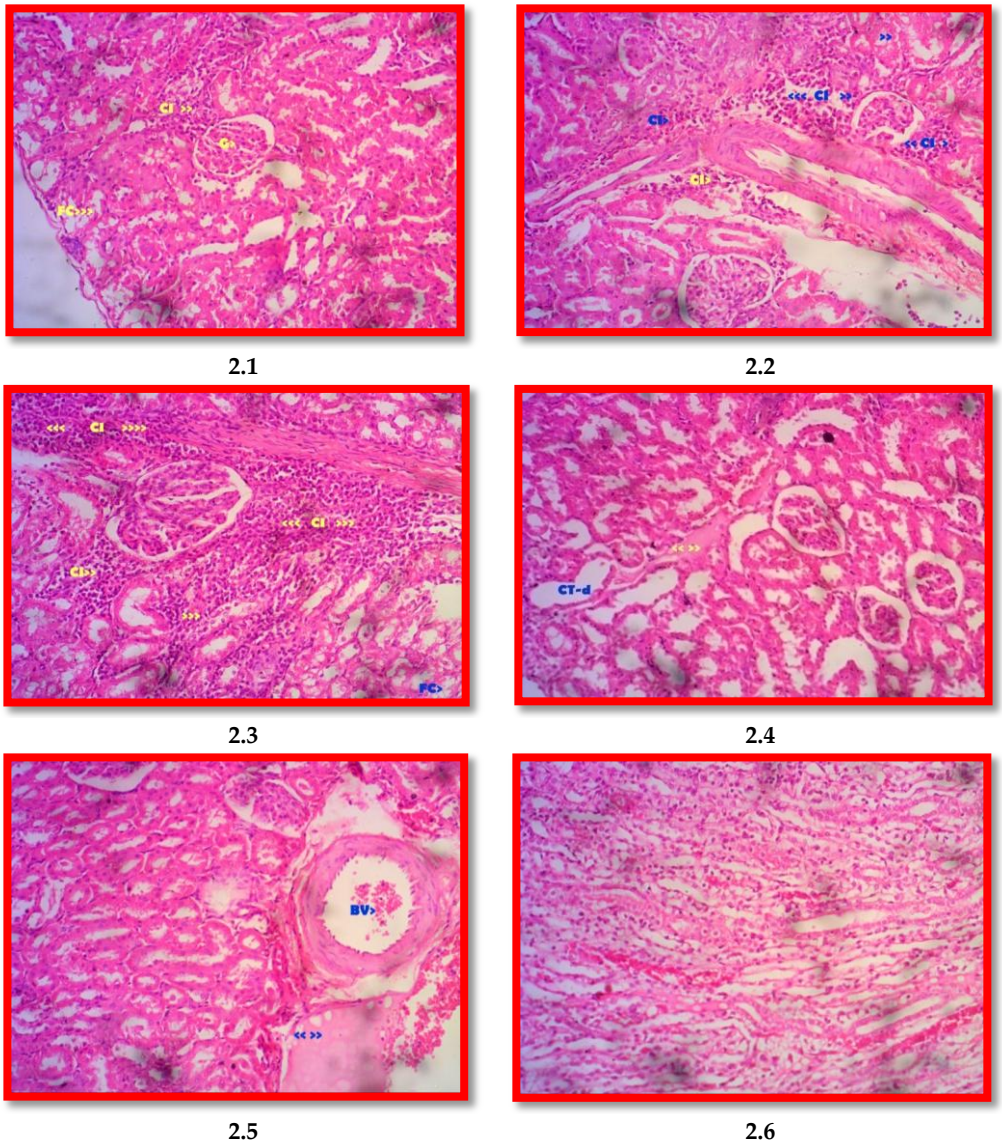
Group	Urine uric acid (mg/100 ml)	% change
Positive control	13266.66 ± 678.56	----
Reference standard (Allopurinol)	3583.33 ± 118.79	72.989 ↓ ##
Test I (*Ashvattha kashaya* – TED)	1374.00 ± 628.28	89.64 ↓ ##
Test II (*Ashvattha kashaya* – TED × 2)	50666.6 ± 10242 ##	281.90 ↑ ##

Data: MEAN ± SEM, **P < 0.01, ##in comparison to positive control.

Histopathology study

Group 2 – Microscopic examination of the kidney sections from potassium oxonate control group revealed tubular dilation, cell infiltration, proteus changes and few crystals in the tubules (Fig. 2).

Figure 2. Histopathological section of kidneys of potassium oxonate induced group



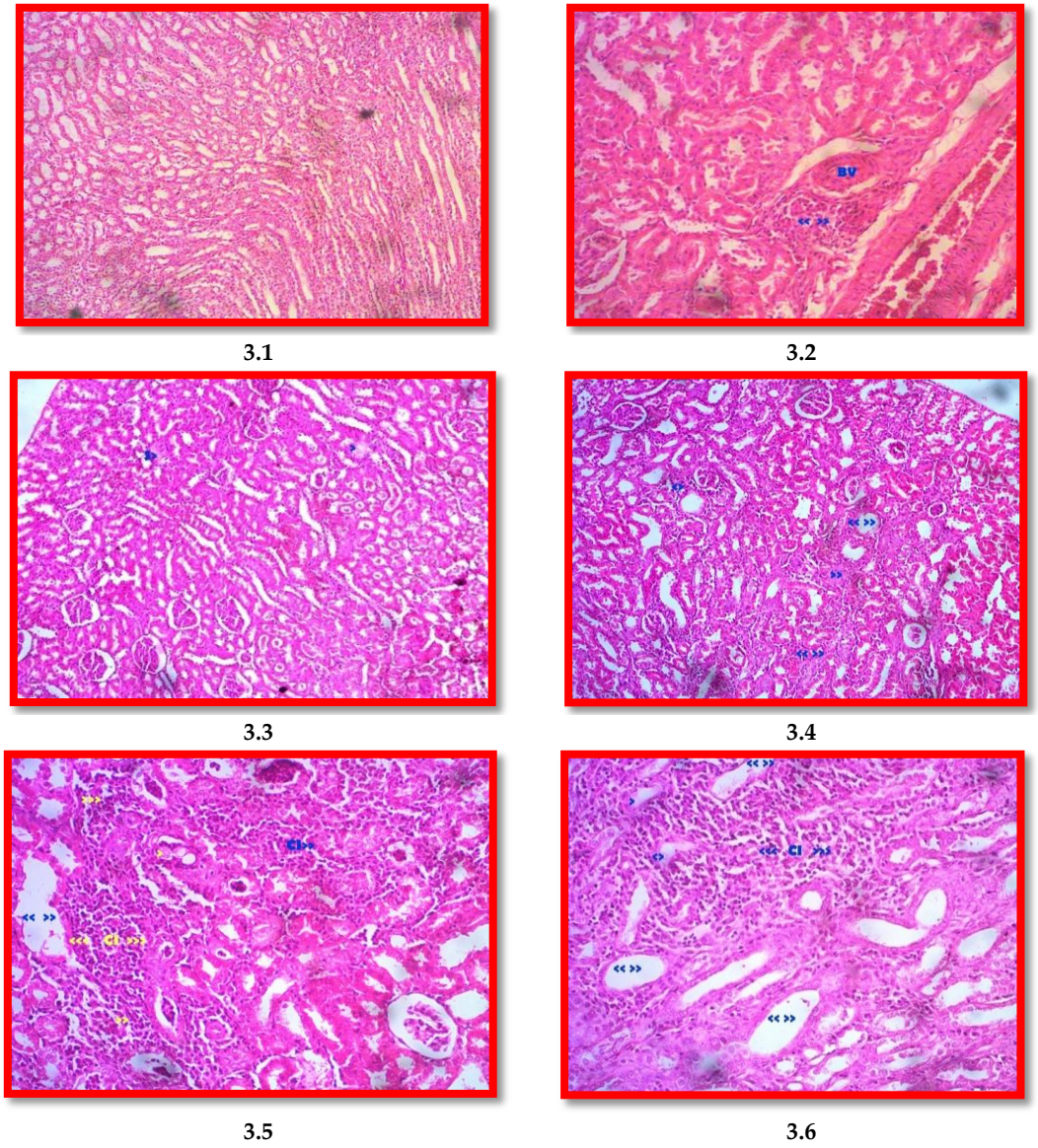
2.1 **2.2**

2.3 **2.4**

2.5 **2.6**

Group 3 – In Allopurinol administered group tubular dilation, cell infiltration, proteus was found to be comparatively less (Fig. 3).

Figure 3. Histopathological section of kidneys of Allopurinol and Potassium oxonate induced group



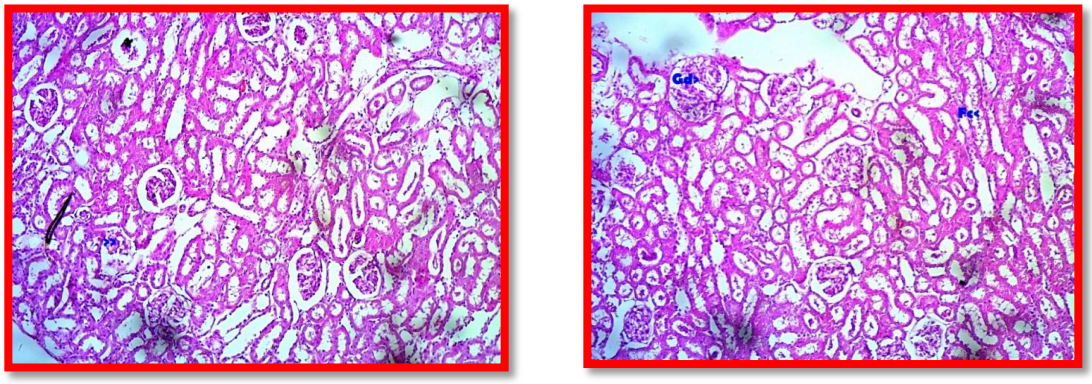
3.1 3.2

3.3 3.4

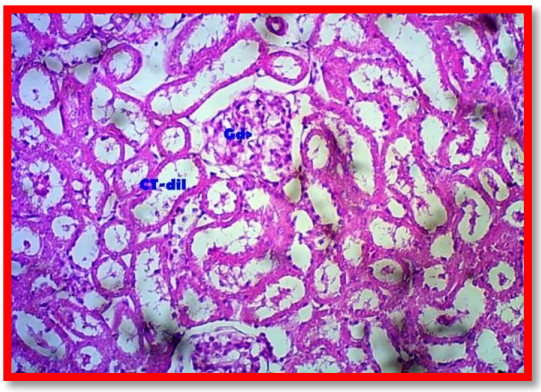
3.5 3.6

Group 4 – Examination of kidney sections from this group exhibited mild to moderate degenerative changes mainly in the form of fatty degenerative changes and tubular dilatation. Glomerular dilation and proteus changes were observed at some sites. Stone formation was mild in comparison to the positive control group (Fig. 4).

Figure 4. Histopathological section of kidneys of Ashvattha kashaya in TED



4.1 4.2



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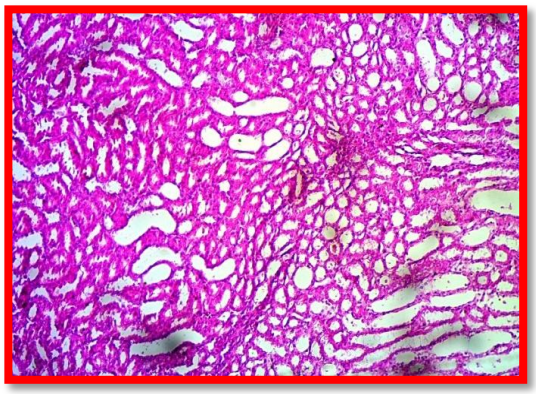

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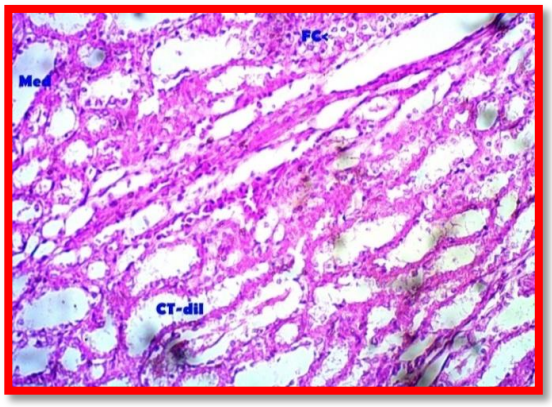

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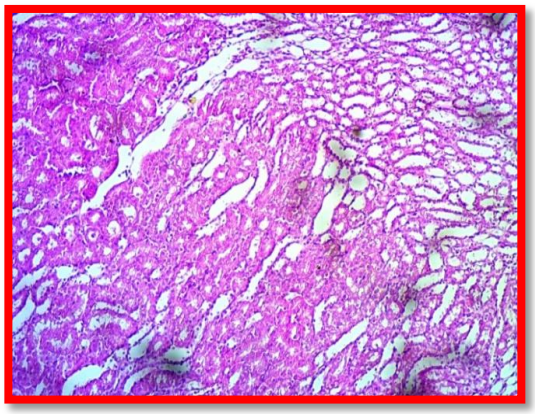
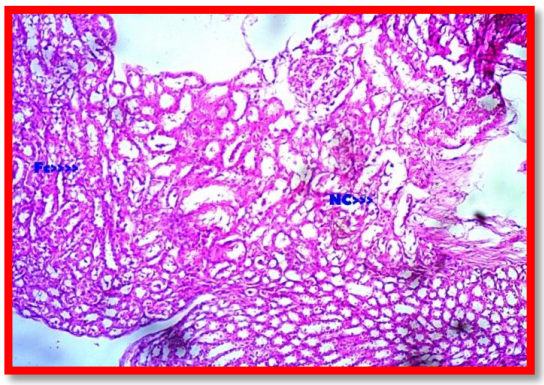


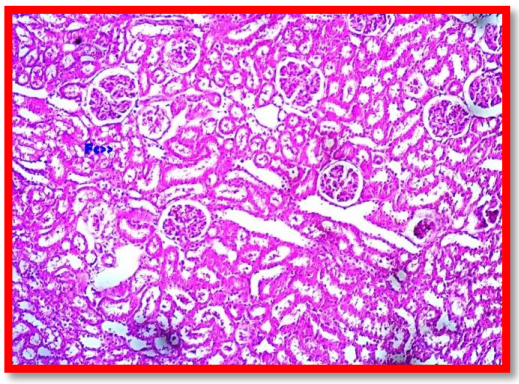
4.6

Group 5 – Examination of kidney sections from this group exhibited mild to, moderate degenerative changes mainly in the form of fatty degenerative changes and tubular dilatation. Stone formation was mild in comparison to the positive control group (Fig. 5).

Figure 5. Histopathological section of kidneys of Ashvattha kashaya in TED × 2



5.1



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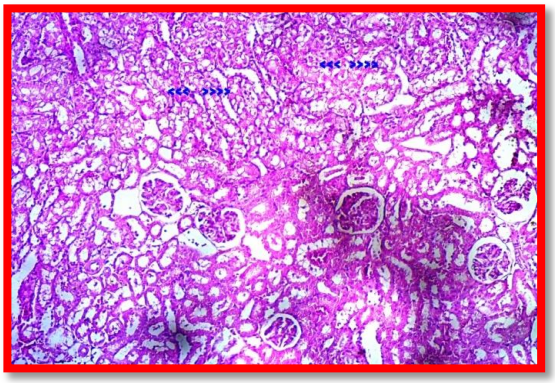

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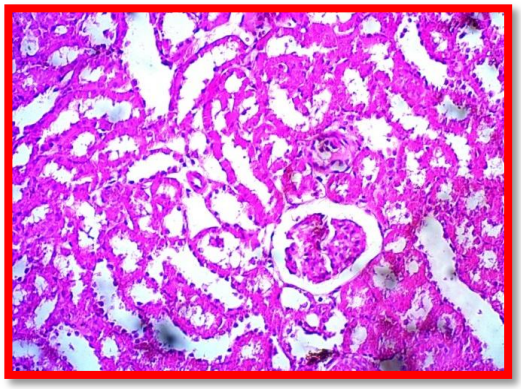

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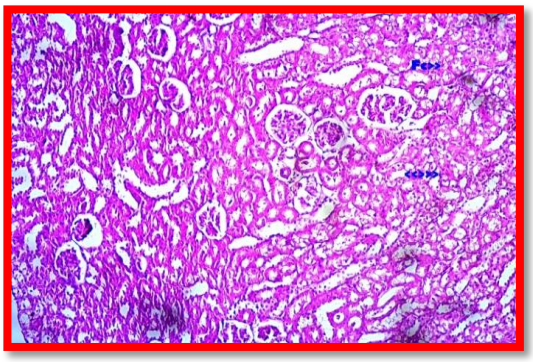

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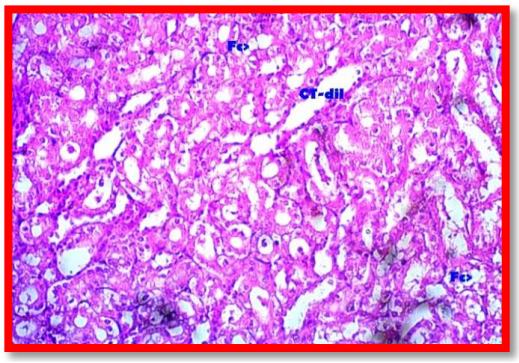

5.6

In the present study, the *kashaya* of bark of *Ashvattha* plant was evaluated for hyperuricemic action at 2 different doses. Potassium oxonate 300 mg/Kg single intra peritoneal injection significantly elevated uric acid level in serum as compared to normal control and it was maintained upto 24 hours. When Potassium oxonate is injected it will act in the uric acid metabolic pathway thus inhibiting uricase enzyme which was indicated by drastic increase in serum uric acid. The kashaya of *Ashvattha* bark is pre-treated for 7 consecutive days' showed non-significant decrease in Potassium oxonate induced hyperuricemia during 3rd hour reading, whereas the test drug in single dose showed significant reduction in the serum uric acid level during 24 hours measurement. This result showed a promising medication to combat the elevated serum uric acid level. The possible mechanism of action might be due to inhibition of xanthine oxidase enzyme (rate limiting enzyme) which is responsible for conversion of Xanthine products to uric acid or it might cause uricosouric action where increased uric acid elimination through urine.

Histological examination revealed that the toxicant induced degenerative changes were only mild to moderate in group 4 in comparison to positive control. Similar but slightly more changes were observed in group 5. This can be taken as evidence for presence of moderate to good nephro-protective effect against the toxicant induced changes.

Ashvattha is a drug which shows *Vichitrapratyarabdhata* properties like *Madhura* and *Kashaya rasa*, *Guru Ruksha guna, Sheeta virya* and *Katu vipaka*. Among these *Madhura rasa* is responsible for the decrease in *vata* and *pitta doshas* and *Kashaya rasa* is having the properties of *rakta prasadana*, *amaharatva* and *mutra sangrahaniya* in its *ruksha bhava*[9]. Here in the present study increased Uric acid crystals were seen in Urine thereby decreasing the level of Uric acid in serum. The *Mutra sangrahaniya* property of *kashaya* may be the cause for this action by detaching the uric acid crystals from the tissue and the same was eliminated through urine, which is considered as one of the *malas* according to Ayurveda. The sheeta virya of the drug will also play an important role in reducing the pitta thereby reduces vitiated rakta. The sheeta virya of the drug have the action to increase the amount of urine and thereby helps to expel the formed uric acid crystals from the body through urine. Guru guna is having the property of vataharatva hence attaining the action accordingly. One of the Purvarupa manifestations of sama vata rakta is shopha. The ruksha guna of Ashvattha can act on shopha in the purvarupa level hence gives good result in Sama vata rakta.

The phyto-constituents present in *Ficus religiosa* Linn. such as tannins, alkaloids and flavonoids has a strong antioxidant, anti-inflammatory and analgesic property[10]. The alkaloid such as colchicine is used in modern system of medicine in treating gout. So the natural plant alkaloid can play an important role in reducing hyperuricemia. Flavonoids have a direct action in xanthine oxidase. Xanthine oxidase metabolism has been implicated as an important route in the oxidative injury to tissues. Both xanthine dehydrogenase and xanthine oxidase are involved in the metabolism of xanthine to uric acid. Flavonoids inhibit xanthine oxidase activity thereby resulting in decreased oxidative injury.

Hence from these findings we can conclude that *Ficus religiosa* bark kashaya can be a potential therapeutic agent and used against vata rakta condition. Since gout is one of the correlation given for gout which is assessed by the parameter hyperuricemia, by uricosuric action the drug will gives good result by expelling the uric acid crystals from the serum by filtration process of kidney.

CONCLUSION

Among all treated group test *Ashvattha kashaya* which was given in double the therapeutic dose on 24th hour had shown extremely significant anti-hyperuricemic activity. The *Mutra sangrahaniya* action of *kashaya rasa* will helps to eliminate the deposited uric acid crystals from the tissues through urine. Hence the study was found to be effective by seeing the observation such as increased uric acid crystals in urine and decreased uric acid in serum. Thus the anti-hyperuricemic activity of *Ashvattha* can be justified.

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CONFLICT OF INTEREST

Nil

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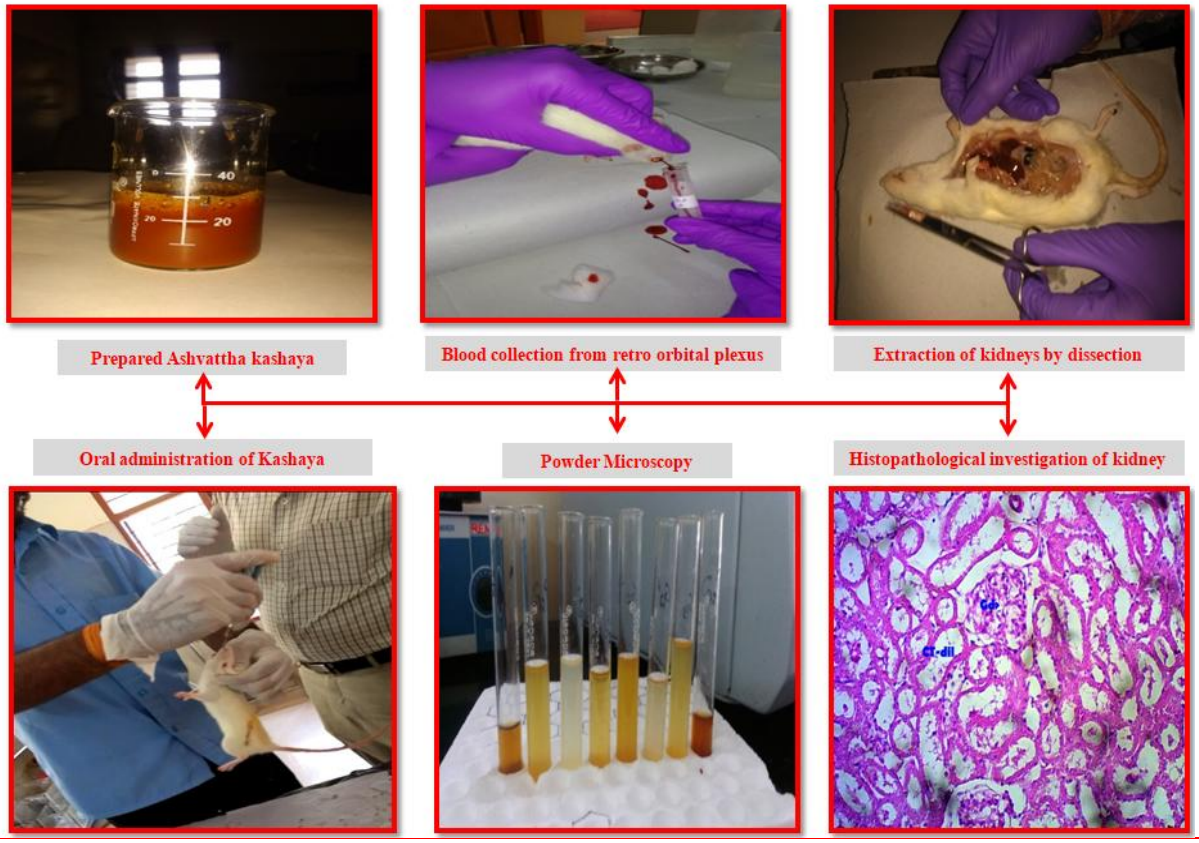
GRAPHICAL ABSTRACT



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