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Research Article

EFFECT OF ETHANOLIC EXTRACT OF *ACALYPHA INDICA* LINN. ON ETHYLENE GLYCOL - INDUCED KIDNEY CALCULI IN RATS

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ABSTRACT

Nephrolithiasis or renal stone disease remains a significant health problem in the adult population. Nephrolithiasis is a recurrent disorder prominent in males. It is significant medical and surgical problem because of incidence, recurrence and severe consequences. The present day medical management of nephrolithiasis is either costly or not without side effects. Invasive procedures for the treatment of nephrolithiasis may cause serious complications and they also impose a great load of costs to the healthcare system. Hence the search for antilithiatic drugs from natural sources has assumed greater importance.The aim of the present study was to evaluate the antilithiatic activity of *Acalypha indica* supplementation on ethylene glycol induced nephrolithiasis in male wistar albino rats. Ethylene glycol feeding resulted in decreased the levels of enzymatic and non-enzymatic antioxidants such as glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G-6-PD), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione, vitamin C and vitamin E in liver and kidney homogenate. Supplementation with ethanolic extract of *Acalyph indica* (200mg/kg b.wt.dose^{-1} day^{-1}oral^{-1}) restored the levels and it brought back the values to near normal range in liver and kidney. The results indicate that the ethanolic extract of *A.Indica* are endowed with significant antiurolithiatic activity.

Keywords: *Acalypha Indica linn.,* Kidney Calculi, Nephrolithiasis.

INTRODUCTION

Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. Urinary calculi have been found in the tombs of Egyptian mummies dating back to 4000BC and in the graves of North American Indians from 1500-1000 BC. Reference to stone formation is made in the early Sanskrit documents in India between 3000 and 2000 BC .Urinary stone diseases has afflicted human kind since antiquity and can persist, with serious medical consequences, throughout a patient's life time. In addition, the incidence of kidney stone has been increased in Western societies in the last five decades, in association with economy development. Most calculi in the urinary system arise from a common component of urine, e.g calcium oxalate [CaOx], representing up to 80% of analyzed stone (Daudon and Jungers, 2001 and Atmani *et al*., 2003).

Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidney. The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Not all standard pharmaceutical drugs used to prevent urolithiasis are effective in all patients, and many have adverse effects that compromise their long-term use. Renal calculi can be broadly classified in two large groups: tissue attached and unattached. Attached calculi are mainly integrated by calcium oxalate monohydrate [COM] renal calculi, with a detectable attachment site to the renal papilla and basically consisting of a core located near to the attachment site [concave zone] and radially striated concentrically laminated peripheral layers. Unattached calculi, with no detectable site of attachment to papilla, are developed in renal cavities of low or reduced urodynamic efficacy and can exhibit diverse composition and structures (Grases *et al.,* 2002 and Low *et al* ., 2000).

The recurrence of urolithiasis is a serious problem, as patient who has formed a stone are more likely to form another and thus stone prevention is highly recommended. Currently open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of extracorporeal shock wave lithotripsy, which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones. However, in addition to the traumatic effects of shock waves, persistent residual stone fragments, and the possibility of infections, suggest that Extra Corporeal Shock Wave Lithotripsy (ESWL) may cause acute renal injury, a decrease in renal function and increase in stone recurrence. Furthermore, although some drugs used to prevent the disease have some positive effect, they are not effective in all patients and often have adverse effects that compromise their use in long term medical treatment (Keller, 1991).

The recent resurgence of plant remedies results from several factors like effectiveness of plant medicines and no side effects compared to modern medicines. In the present scenario, the need for basic scientific investigations on medicinal plants used in the indigenous systems becomes imminent. This is evident by the increase in number of reports by various investigators supporting the claims of medicinal plants and a dramatic increase in the share of plant products in pharmaceutical market (Prasad *et al*., 2007).

Acalypha indica is a small erect herb up to 60 cm tall or a little more, with a few ascending branches, these angled and pubescent; leaves broadly ovate, subdeltoid, rather coarsely toothed, on petioles as long as or longer than the 3-5 cm long blades; nerves 3-5 from base, thereafter pinnately arranged; stipules minute; flowers sessile on erect axillary spikes longer than the leaf; male flowers minute, crowded distally; stamens 8, female flowers scattered along the inflorescence axis, each subtended by a conspicuous semicupular foliaceous toothed green bract nearly mm long; capsule hispid, 1 mm broad, 3-locular (Stone and Benjamin,1970).The present study aims to give data highlighting the present trends in research of medicinal plants accredited with antiurolithiatic activity. This may help investigators to identify and develop appropriate lead compounds or plant products beneficial in the management of urolithiasis.

MATERIALS AND METHODS

Collection of the plant material

Acalypha indica was collected from Maruthamalai hills, Coimbatore district, Tamil nadu, India during the month of March to May, 2009. The plant was identified and authenticated by Taxonomist Dr.K.Arumugasamy, Lecturer (SG), Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Selection of animals

For the purpose of urolithiatic studies, adult male wistar albino rats weighing about 150 to 200 g were collected from animal breeding centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hours light and dark cycle at 28^{o}C ± 2^{o} C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines suggested by the institutional animal ethics committee (IAEC).

Stone Induction

Animals were fed with 0.75% (0.75ml of ethylene glycol in 100 ml of drinking water) of rats for a period of 30 days to induce urolithiasis. The urolithiatic rats were then used for the study.

Table 1: Experimental design of animals for antiurolithiatic activity

Group	Experimental design
I	**Control rats**- received normal pelleted diet
II	**Urolithiatic rats** - Urolithiasis was induced by administrating0.75% of ethylene glycol in 100ml of drinking water to rats for 30 days
III	**Treated rats**– Urolithiatic rats received ethanolic extract of *A. indica* (200 mg / kg body wt) by oral administration for 30 days at a rate of 1.0 ml / rat / day.
IV	**Standard drug thiazide treated rats**– Urolithiatic rats received thiazide (150µg/ kg body wt) by oral administration for 30 days at the rate of 1.0 ml / rat / day.

The rats were divided into four groups of six animals each.

Collection of liver and kidney samples

The experimental animals were sacrificed, liver and kidney were removed immediately, washed with ice cold saline and their weights were recorded. Small pieces of liver and kidney tissues were collected in 10% formalin and used for histopathological studies.

Chemicals

All chemicals used in the present study were of analytical reagent grade.

Statistical Analysis

The results of the biochemical estimations were reported as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by one way Analysis Of Variance (ANOVA) followed by the analysis of level of significance between different groups based on ANOVA using AGRES statistical package (Version 3.1). Difference among means were analysed by least significant difference (LSD) at 5% level ($p<0.05$).

RESULTS AND DISCUSSION

Table 1, 2, 3 and 4 represent the activities of enzymatic antioxidants such as glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G-6-PD), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and in kidney and liver of control and experimental rats.

From the representations it is evident that the levels of enzymatic antioxidants in kidney and liver were significantly decreased ($p < 0.05$) in urolithiatic rats (Group II) when compared to control rats (Group I). The above alterations were reverted to near control in rats treated with plant extract group III rats. When *A.indica* extract treated rats (Group III) were compared with thiazide treated rats (Group IV) there was no significant difference between these groups of rats. This result gives a supportive evidence for the antiurolithiatic activity of ethanolic extract similar to standard drug thiazide**.**

Table 2: Effect of *A.indica* extract on the levels of GST, G-6-PD and GR in kidney of control and experimental rats

Group	GST$^{\lozenge}$	G-6-PD$^{\lozenge\lozenge}$	GR$^{\lozenge\lozenge\lozenge}$
I	8.01 ± 0.44	9.10 ± 0.06	45.45 ± 0.24
II	2.37 ± 1.18 a*	2.01 ± 0.89 a*	36.63 ± 1.76 a*
III	7.20 ± 0.52 b*,d^{ns}	8.36 ± 0.18 b*,d^{ns}	43.68 ± 0.44 b*,d^{ns}
IV	7.51 ± 0.36 c*	8.42 ± 0.13 c*	44.62 ± 0.24 c*

Values are expressed as mean ± SD of six animals

Experimental design

Group I : Control rats – received normal pelleted diet

Group II : Received 0.75% ethylene glycol in water for 30days

Group III : Treated rats –Urolithiasis induced rats received *A.indica* extract (200 mg / kg body weight) by oral administration for 30 days at a rate of 1.0 ml / rat / day

Group IV : Standard drug thiazide treated rats – Urolithiasis induced rats received thiazide (150 µg / kg body weight) by oral administration for 30 days at a rate of 1.0 ml / rat / day.

Comparison between the groups

'a' represents comparison between II and I

'b' represents comparison between III and II

'c' represents comparison between IV and II

'd' represents comparison between III and IV

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

$\lozenge$ µg of CDNB-GSH conjugate /min/mg protein

$\lozenge\lozenge$ 0.01 OD /min/mg protein

$\lozenge\lozenge\lozenge$ n moles of NADPH broken /min/mg protein

Table 3: Effect of *A.indica* extract on the levels of SOD, CAT and GPx in kidney of control and experimental rats

Group	SOD$^{\text{¤¤}}$	CAT$^{\#\#\#}$	GPx$^{\text{¤¤}}$
I	3.11± 0.20	26.13 ± 0.57	18.25 ± 0.17
II	2.14 ± 1.02 a*	18.48 ± 1.63 a*	5.79 ± 1.21 a*
III	2.67 ± 0.21 *,d^{ns}	24.77 ± 0.51 b*,d^{ns}	17.19 ± 0.12 b*,d^{ns}
IV	2.86 ± 0.16 c*	24.95 ± 0.47 c*	17.15 ± 0.07 c*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 2

The symbols represent statistical significance p* < 0.05, ns – not significant.

Units

✪✪50% inhibition of nitrate formation / min / mg protein

µmoles of hydrogen peroxide decomposed / min / mg protein

✇✇µg of glutathione consumed / min / mg protein

Table 4: Effect of *A.indica* extract on the levels of GST, G-6-PD and GR in liver of control and experimental rats

Group	GST◊	G-6-PD◊◊	GR◊◊◊
I	7.17 ± 0.12	7.26 ± 0.39	42.26 ± 0.53
II	3.22 ± 0.54a*	2.75 ± 1.17 a*	35.45 ± 1.91 a*
III	6.22 ± 0.19 b*,d^{ns}	7.00 ± 0.44 b*,d^{ns}	38.38 ± 0.65 b*,d^{ns}
IV	6.43 ± 0.16 c*	7.19 ± 0.33 c*	38.83 ± 0.46 c*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 2

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

◊ µg of CDNB-GSH conjugate /min/mg protein

◊◊ 0.01 OD /min/mg protein

◊◊◊ n moles of NADPH broken /min/mg protein

Table 5: Effect of *Acalypha indica* extract on enzymatic antioxidants in liver of control and experimental rats

Group	SOD✪✪	CAT###	GPx✇✇
I	7.09 ± 0.08	34.49 ± 0.70	7.23 ± 0.11
II	2.50 ± 1.12a*	27.00 ± 1.95 a*	3.28 ± 1.30 a*
III	7.07 ± 0.16 b*,d^{ns}	33.43 ± 0.73 b*,d^{ns}	6.78 ± 0.27 b*,d^{ns}
IV	7.20 ± 0.14 c*	33.79 ± 0.51 c*	6.79 ± 0.26 c*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table2

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

✪✪50% inhibition of nitrate formation / min / mg protein

µmoles of hydrogen peroxide decomposed / min / mg protein

✇✇µg of glutathione consumed / min / mg protein

Table 5 and 6 the activities of the non-enzymatic antioxidants such as total reduced glutathione, vitamin C and vitamin E in liver and kidney of control and experimental rats.

From the table 5& 6 it is evident that the levels of non-enzymatic antioxidants were significantly decreased (p< 0.05) in urolithiatic rats (Group II) when compared to control rats (Group I). Treatment with plant extract formulation for group II rats restored the levels and it brought back the values to near normal range in group III rats. When *A.indica* extract treated rats (group III) were compared with thiazide treated rats (Group IV), there was no significant difference between these groups of rats. This result gives a supportive evidence for the antiurolithiatic activity of ethanolic extract similar to standard drug thiazide**.** . This result gives a supportive evidence for the antiurolithiatic activity of ethanolic extract similar to standard drug thiazide**.**

Table 6: Effect of *Acalypha indica* extract on non-enzymatic antioxidants in liver of control and urolithiatic rats

Group	GSH¥	Vitamin C¥	Vitamin E¥
I	18.27 ± 0.12	6.44 ±0.24	10.46 ±0.23
II	11.71 ± 1.40 a*	3.66 ±1.26 a*	5.12 1±.02 a*
III	17.26 ± 0.19 b*,d^{ns}	17.26 ± 0.19 b*,d^{ns}	9.48 0±.23 b*,d^{ns}
IV	17.27 ± 0.15 c*	6.01± 0.27 c*	9.39 ±0.20 c*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table2

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

¥µg /mg protein

Table 7: Effect of *Acalypha indica* extract on non-enzymatic antioxidants in kidney of control and urolithiatic rats

Group	GSH¥	Vitamin C¥	Vitamin E¥
I	20.51 ± 0.18	8.29 ± 0.16	13.23± 0.17
II	14.56 ± 1.30 a*	2.78 ± 1.01 a*	8.17± 1.05 a*
III	19.29 ± 0.19 b*,d^{ns}	7.50 ± 0.22 b*,d^{ns}	12.92± 0.1 b*,d^{ns}
IV	19.36 ± 0.16 c*	7.46 ± 0.22 c*	13.29 ±0.19 c*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table2

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

¥µg /mg protein

DISCUSSION

Superoxide dismutase (SOD) is widely distributed in cells with high oxidative metabolism and has been proposed to protect against the deleterious effect of superoxide anion. SOD catalytically scavenges the superoxide radicals and thus renders cytoprotection against free radical damage (Fridovich, 1975).Catalase and glutathione peroxidase are involved in the elimination of hydrogen peroxide. The decreased activity of glutathione peroxidase may be correlated to decreased availability of its substrate reduced glutathione. Lowered levels of reduced glutathione have been reported in urolithiatic rats (Muthukumar and Selvam, 1998). Similar results were got in our studies also.Antioxidant can be classified as preventive and chain breaking antioxidants. Antioxidant vitamin such as α-tocopherol (vitamin E) belongs to the second category. These compounds can intercept free radical induced chain reaction and prevent further oxidation. Crystal adherence to the surface of injured renal epithelial cells is considered initiating event in the genesis of urolithiasis. However, the oxidant (free radical production) and antioxidant imbalance may be one of the major factors leading to the process of crystal deposition in renal tissues (Kato *et al*., 2007).

Our results coincide with that of Kim *et al.* (2004) who showed that *Camelia sinensis* (Green tea) and *Aspalathus linearis* (rooibos tea) both has inhibitory effect on kidney stone formation with increased antioxidant activity in rats.

Our results coincide with that of Farooq *et al.* (2004) who showed that the levels of antioxidants significantly increased by the administration of phycocyanin from *Spirulina platensis.*

Veena *et al.* (2005) showed that the sulfated polysaccharides from edible sea weed *Fucus vesiculosus* increased the antioxidant levels in experimental hyper oxaluria. Thus from the above results it was evident that the levels of non enzymatic antioxidant were brought back to near normal levels on treatment with poly herbal formulation.

This study suggests that *A.Indica* could prevent the free radical formation from calcium oxalate urolithiasis in rats and thus protecting the renal cells from oxidative injury.

CONCLUSION

In conclusion, the result of our study clearly revealed that the ethanolic extract of *Acalypha indica* have potent antiurolithiatic activities in ethylene glycol induced male wistar albino rats. Our results show that the anti urolithiatic effect of the plant may be due to its antioxidant, free radical scavenging properties of the secondary metabolites present in the plant.

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