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NEPHROPROTECTIVE ACTIVITYOF *ACORUS CALAMUS* LEAVES EXTRACT AGAINST LITHIUM INDUCED NEPHROTOXICITY IN WISTAR RATS

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Abstract:

Acorus calamus is a tall perennial herb. It is a wetland monocot of the Acoraceae family, in the genus Acorus.It is also called sweet flag or calamus. Sweet Sedge is 30 to 100 cm tall. Tufts of basal leaves arises from a spreading rhizome. Calamus is used as sedative, diuretic, laxative and as a carminative agent in modern herbal science medicines. Its use in siddha and ayurvedic medicine is also reported where in stems, leaves, roots, barks plays magnificent role. Few uses approved for calamus is as a flavour for pipe tobacco from the rhizome essence and is also used as essential oil in perfume industry. The objective of present study is to evaluate the nephroprotective activity of ethanolic extract of leaves of Acorus calamus at the dose of 500mg/kg body weight in wistar rats and to carry out phytochemical screening of leaves extract, measure serum urea, creatinine, blood urea nitrogen (BUN), albumin and uric acid.And to describe the histopathological status of kidneys in the treated and untreated groups. Nephroprotective activity was evaluated against lithium chloride induced nephrotoxicity in rats. Phytochemical screening of the extract was done. 24 Animals were divided into 4 groups each containing 6 rats. Group I received normal saline 5ml/kg for 5 weeks. Group II is treated with nephrotoxicant i.e., 1ml/kg of 15% Licl solution for 1 week + tap water for 1 week + 7% Licl for 2 weeks + tap water for 1 week. Group III is fed with toxicant (Licl solution as above) for 5 weeks &A.calamus leaves extract 500mg/kg for 5 weeks which is considered as test .Group IV is given toxicant as in case of group II with standard drug named Cystone 5ml/kg for 5 weeks. Samples of kidney tissue were removed for histopathological examination. Screening of Acorus calamus showed the presence of nephroprotective phytochemicals. Ethanolic extract of leaves produced significant nephroprotective activity in lithium chloride induced nephrotoxicity which was evident by decreased levels of serum urea, creatinine, BUN and uric acid. Acorus calamus contains nephroprotective phytochemicals and may be useful in preventing kidney damage induced by Licl.

Key Words: *Acorus calamus; lithium chloride; nephroprotective activity*

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INTRODUCTION:

For treating persons with bipolar depression, lithium is currently the drug of choice and is widely used in this population. For psychiatric problems, approximately 0.1% of US population is undergoing lithium treatment. At least one episode of lithium toxicity is observed by approximately 30% of patients taking lithium. The chronic lithium nephrotoxicity is dominated by polyuria and evidence of chronic kidney disease.[32]The acute lithium nephrotoxicity picture is dominated by evidence of volume depletion, obtundation, and the potential for cardiovascular collapse. Correction of electrolyte abnormalities, volume depletion followed by forced diuresis, and dialysis in severe cases are involved in treating acute lithium toxicities. In patients with chronic toxicity, with the routine measures used for CKD, chronic renal insufficiency can be treated ,[32] and polyuria can be treated with medications [1-7].

Pathophysiology of lithium nephrotoxicity

In the metal series, Lithium is a univalent cation. It doesn't have any role in human physiology but is closely related to both sodium and potassium. Lithium is completely absorbed by the GI tract. The drug is not protein bound and is completely filtered at the glomerulus. [35] Most of the filtered load is reabsorbed by the proximal tubule, but significant amounts are also absorbed in the loop of Henle and the early distal nephron. Up to 90% of the filtered load is reabsorbed by the nephron, 60% in the proximal tubule, and the remainder in the thick ascending limb of the loo of Henle, the connecting tubule, and the cortical collecting duct. Lithium can substitute for sodium in several sodium channels, particularly the sodium-hydrogen exchanger in the proximal tubule (NHE3), the sodium/potassium/2chloride exchanger in the thick ascending limb of the loop of Henle (NKCC2), and the epithelial channel of the cortical collecting tubule (ENaC).[36] Lithium can affect renal function in several ways. Acutely and chronically, lithium salts produce a natriuresis that is associated with an impaired regulation of the expression of the epithelial sodium channel in the cortical collecting tubule. Specifically, the ability of aldosterone to increase apical membrane ENaC expression may be inhibited partially, resulting in inappropriate sodium losses [7-9].

Nephrogenic diabetes insipidus the most common complication of long-term lithium therapy. At the cellular level, antidiuretic hormone (ADH) is released from the posterior pituitary in response to increases in serum osmolarity or decreases in effective circulating volume, and this hormone acts on V2 receptors in the basolateral membrane of the principal cells in the cortical and medullary collecting tubules. An increase in the intracellular cyclic adenosine monophosphate (cAMP) level is because of the result of the cascade involving a G protein (guanylyl-nucleotide regulatory protein) and adenylate cyclise, which can play a dual role in antidiuretic regulation. Protein kinase A is stimulated by cAMP, which facilitates the insertion of aquaporin-2 (AQP2) water channels. These water channels are preformed and stored in cytoplasmic vesicles in the apical plasma membrane of the principal cells. This process leads to increased water permeability and, thus antidiuresis.[37] At the genetic level by promoting a 5' untranslated region of the *AQP2* gene[36.] cAMP levels increase which also increase the production of AQP2 water channels [10,11].

Signs and symptoms of lithium nephrotoxicity:

1) Gastrointestinal pain or discomfort, diarrhoea, tremor, polyuria, nocturnal urination, weight gain, oedema, flattening of affect and exacerbation of psoriasis are typical complaints of patients receiving long-term lithium therapy.
2) Lithium nephrotoxicity results in a reduced urinary concentrating capacity, expressed as obligate polyuria, with secondary thirst. With long-term therapy of lithium, this may result in nephrogenic diabetes insipidus.[39]
3) In addition, glomerular filtration rate falls slightly in about 20% of patients. Patients on long-term lithium therapy are at increased risk of glomerular impairment and progressive renal insufficiency.[40]
4) Lithium treatment may inhibit thyroid hormone release and induce goitre. Consequently, the prevalence of both overt and subclinical hypothyroidism is increased.
5) Long-term lithium treatment may be associated with persistent hyperparathyroidism and hypercalcaemia, as well as with hypermagnesemia. Overweight of up to 4–10 kg is observed in around 30% lithium treated patients.
6) Cognitive disturbances are often mentioned as a lithium-related adverse effect. [39]
7) Many deaths have been reported with lithium treatment. Non-accidental overdose (either long-term over dosage or acute overdose on long-term treatment) is the most serious issue. Exceptional complication of long term lithium therapy is progressive renal insufficiency which has fatal outcome.[38]
8) In relation to pregnancy, lithium salts are rated as category D (positive evidence of risk).

Therefore, prescription of lithium should be avoided during the first trimester of pregnancy unless the benefit to the mother exceeds the risk to the foetus.

9) It is a controversial topic whether lithium therapy is safe in pregnant women or not, although lithium transfer into breast milk is well established [11-15].

METHODOLOGY:

MATERIALS-

Plant collection and authentication

The *Acorus calamus* plant was authenticated by Dr. Sadia Fatima, dept. Of Botany, Anwarul Uloom College of Pharmacy. It was collected from Central Research Institute of Unani Medicines (CRIUM), Erragadda, Hyderabad.

Preparation of plant extract:

100g Leaves were air dried and shade dried for 2 days and then dried in hot air oven at 25ºC for 3 days and they were made into coarse powder with the use of mixer grinder, the powder of entire plant of *Acorus calamus* obtained were weighed separately and transferred to a round bottomed flask and macerated with ethanol for 7 days. Solvent was filtered and concentrated by rotary vacuum evaporator. Extract was stored at 4ºC until use. The percentage yield of extract was 18%. A stock solution of the dried powder was reconstituted in distilled water at a concentration of 500mg/ml and administered orally to the animals.

Drugs:

Lithium chloride (from dabur pharma Ltd)

Chemicals :

Anaesthetic ether –SD finechem Ltd., Mumbai , Chloroform – SD finechem Ltd ., Mumbai, Formalin – SD finechem Ltd ., Mumbai , Hydrochloric acid – SD finechem Ltd ., Mumbai , Sodium hydroxide pellets – SD Finechem Ltd., Mumbai , Ethanol –SD Finechem Ltd., Mumbai , Haematoxylin-eosin dye – SD Finechem Ltd., Mumbai ,99m technetium diethylaminepentaacetic acid (^{99m}Tc) – SD Finechem Ltd., Mumbai ,Alpha naphthol solution – SD Finechem Ltd., Mumbai, Sodium nitroprusside solution – SD Finechem Ltd., Mumbai , Lead acetate and glacial acetic acid – SD Finechem Ltd., Mumbai , all the chemicals mentioned above were procured from SD Fine chemicals and were of analytical grade. Reagents like Millions reagent, Ninhydrin reagent, Myer's reagent, Hager's reagent, Wagner's reagent etc. were prepared according to the need and some were purchased from commercial sources.

Diagnostic kits:

Diagnostic kits used for estimation of calcium, sodium, potassium, chloride, uric acid and urea, creatinine, phosphorus, total protein, albumin (were procured from Span Diagnostic Ltd India)

Instruments:

Autoanalyzer (star21plus), refrigerator centrifuge (MPW- 350R), UV-Spectrophotometer (UV-1601, Shimadzu corporation, Kyoto, japan), Mini Lyotrap (LTE Scientific Ltd.,), Research Centrifuge (Remi industries, Mumbai) and Homogenizer (Remi Motors, Mumbai). Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India), Hot air oven (Lab Hosp, KW-800W, Mumbai).

Experimental Animals:

Wistar rats (weighing 150 - 180 g) of male sex were obtained from the central animal house of Nizam Institute of Pharmacy, Deshmukhi, Yadadri Bhuvanagiri. They were housed in standard metal cages. They were provided standard pellet diet and water. They were allowed a one-week acclimatization period prior to the study. The Animals are maintained under standard laboratory conditions (i.e., 12:12 hour light & dark sequence, at an ambient temperature of 25±2ºC, 35-60% Humidity). The equipment, handling and sacrificing of the animals were in accordance with the Animal Ethics committee for protection of animals.

METHODOLOGY

NEPHROPROTECTIVE STUDIES

24 Animals were divided into 4 groups each containing 6 rats. The lithium induced nephrotoxicity (moderate renal failure) in rat model is used for the evaluation of the nephroprotective activity of the selected medicinal plant -leaves ethanolic extract.

Induction of renal failure:

Study included 24, 2month old, wistar male rats weighing 150-180g. Renal failure was induced by **lithium chloride** according to following protocol: table 1

Table 1. Administration of plant extracts and drugs to the rats

S.NO	GROUP	TYPE OF GROUP	DRUG USED	QUANTITY ADMINISTERED
1	Group I	Normal	Normal saline for 5 weeks	5 ml/kg
2	Group II	Disease	15% Licl (solution given orally as a drinking substance) for 1 week + tap water for 1 week +7% Licl for 2 weeks + tap water for a1 week	1ml/kg
3	Group III	Test	15% Licl (solution given orally as a drinking substance) for 1 week + tap water for 1 week +7% Licl for 2 weeks + tap water for 1 week & *A.calamus* extract for 5 weeks	1ml/kg 500 mg/kg
4	Group IV	standard drug	15% Licl (solution given orally as a drinking substance) for 1 week + tap water for 1 week +7% Licl for 2 weeks + tap water for 1week &Cystone syrup for 5 weeks	1ml/kg 5ml/kg

Group I received normal saline 5ml/kg for 5 weeks. Group II is treated with nephrotoxicant i.e., 1ml/kg of 15% Licl solution for 1 week + tap water for 1 week + 7% Licl for 2 weeks + tap water for 1 week. Group III is fed with toxicant (Licl solution as above) for 5 weeks & *A.calamus* leaves extract 500mg/kg for 5 weeks which is considered as test. Group IV is given toxicant as in case of group II with standard drug named Cystone 5ml/kg for 5 weeks.

In week 6, the glomerular filtration rate (GFR) was assessed by 99m Technetium diethylenetriaminepenta acetic acid, and serum creatinine, blood urea nitrogen (BUN), uric acid, urine volume, urine sodium and potassium and urine creatinine were measured. After GFR evaluation ,8 rats (two from each group) were selected randomly, were sacrificed by halothane overdose, there kidneys were excised for pathological evaluation, preserved in formalin, and embedded in paraffin. Large sections (1 mm) were cut perpendicularly to the renal capsule, to ensure that both cortex and medulla would be present in each section. Samples were stained with haematoxylin–eosin dye.

RESULTS:

The phytoconstituents of *Acorus calamus* were found to be carbohydrates, glycosides, alkaloids and flavonoids. The results of preliminary studies of the plant extract are presented in table 2.

Table 2 - Results of preliminary studies of plant extracts -

PHYTOCONSTITUENTS	PRESENT OR ABSENT
Carbohydrates	+
Glycosides	+
Alkaloids	
1.Mayer's test	-
2.Wagner's test	+
3.Dragendroff's test	-
Saponins	-
Steroids	-
Tannins	-
Flavonoids	+

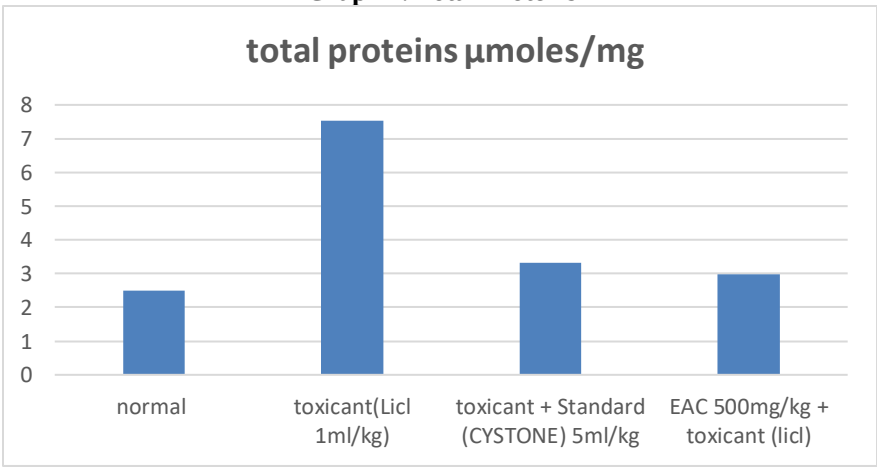
+ = Present
- = Absent

EFFECT OF *Acorus calamus* ON SERUM PARAMETERS IN LITHIUM CHLORIDE INDUCED NEPHROTOXICITY RATS

Total protein

The serum total protein (mg/dl) level of normal, control and treated was shown in Table 6. The serum total protein of normal group-I were in normal range. In group-II serum total protein were significantly (P<0.001 Vs normal) increased. The treatment with Cystone 5ml/kg in group-IV and ethanolic extract of *Acorus calamus* (EAC) at dose 500mg/kg, orally to group-III were significantly (P<0.05-0.001) decreased, when compared to group-I. The serum total protein level at 500mg/kg was reduced near to cystone.

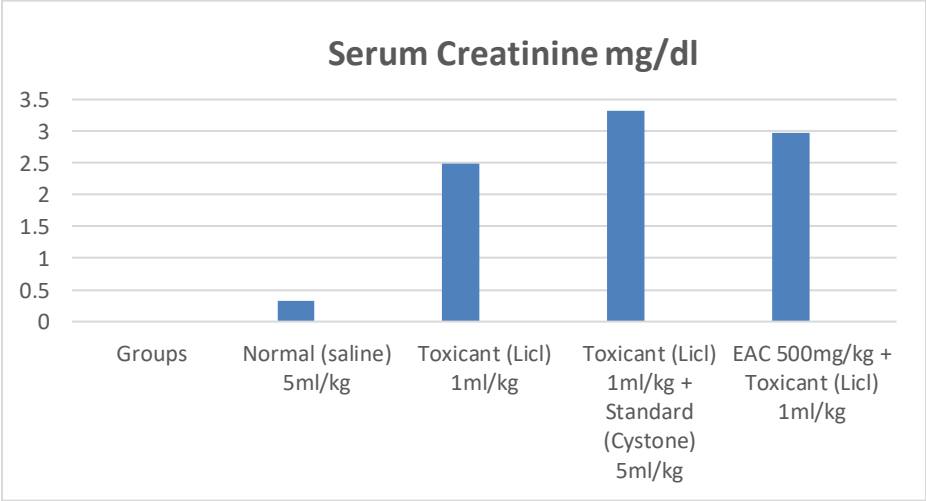
Graph 1. Total Proteins



Creatinine

The serum creatinine (mg/dl) level of normal, control and treated was shown in Table 6. The serum creatinine level of normal group-I were in normal range.. In group-II serum creatinine were significantly (P<0.001 Vs normal) increased. The treatment with Cystone 5ml/kg in group-IV and ethanolic extract of *Acorus calamus* (EAC) at dose 500mg/kg, orally to group-III were significantly (P<0.05-0.001) decreased, when compared to group-I. The serum creatinine level at 500mg/kg was reduced near to cystone.

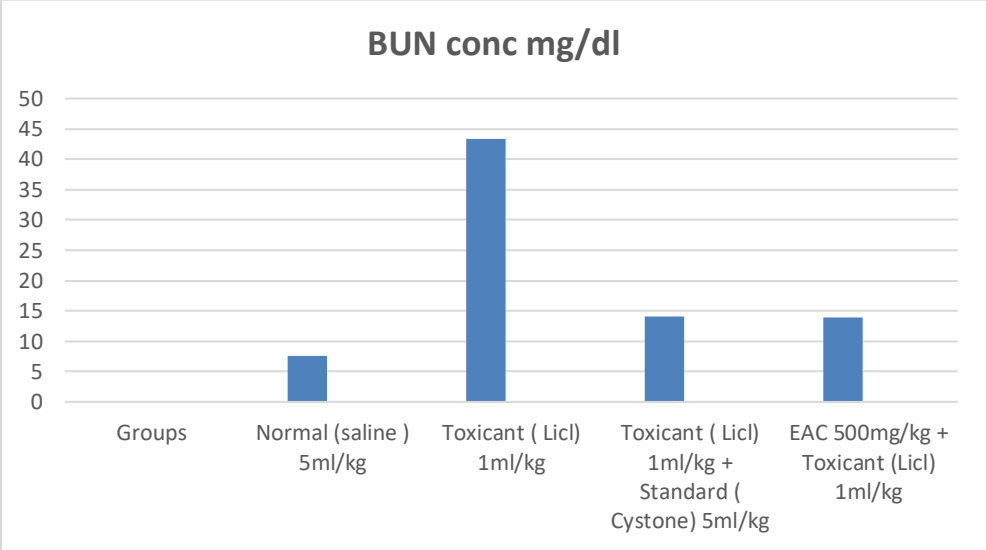
Graph 2. Creatinine



Blood Urea Nitrogen (BUN)

There was increase in the BUN level after the administration of lithium chloride in the normal group when compared (P<0.001) to the normal animals indicating nephrotoxicity. Administration of Cystone 5ml/kg in group-IV and ethanolic extract of *Acorus calamus* (EAC) at dose 500mg/kg, orally to group-III were significantly (P<0.001) decreased in BUN level when compared to group-I as indicated in table 6

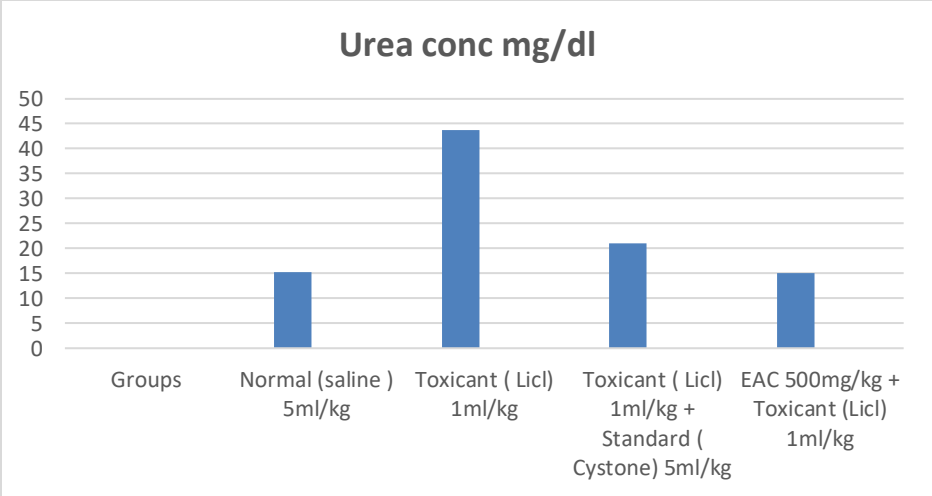
Graph 3. BUN



Urea

The serum urea (mg/dl) level of normal, control and treated was shown in Table 6. The serum urea level of normal group-I were in normal range.. In group-II serum urea were significantly (P<0.001 Vs normal) increased. The treatment with Cystone 5ml/kg in group-IV and ethanolic extract of *Acorus calamus* (EAC) at dose 500mg/kg, orally to group-III were significantly (P<0.001) decreased, when compared to group-I. The serum urea level at 500mg/kg was reduced near to cystone.

Graph 4. Urea



EFFECT OF *Acorus calamus* ON CHANGE IN BODY WEIGHT AND RELATIVE KIDNEY WEIGHT IN LITHIUM CHLORIDE INDUCED NEPHROTOXICITY IN RATS

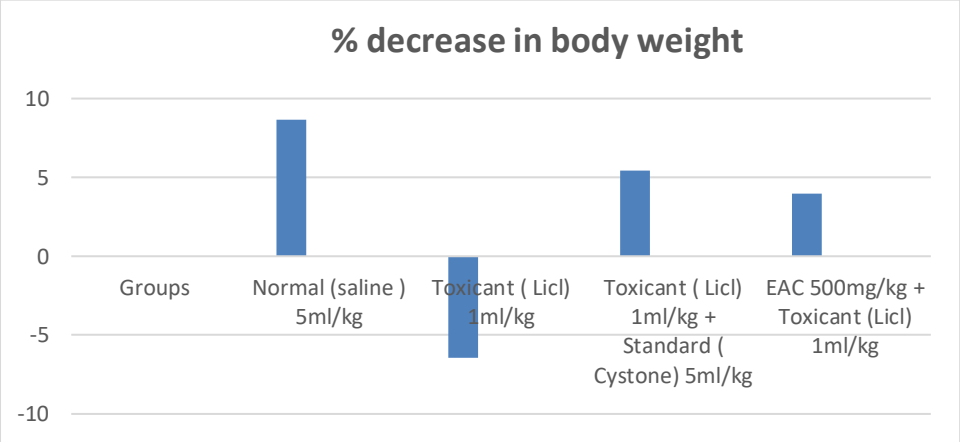
Change in body weight

The change in body weight (%) of normal, control and treated was shown in Table 6. In group-II change in body weight were significantly (P<0.001 vs normal) decreased. The treatment with Cystone 5ml/kg in group-IV and ethanolic extract of *Acorus calamus* (EAC) at dose 500mg/kg, orally to group-III were significantly (P<0.001) increased, when compared to group-I. The change in body weight at 500mg/kg was increased near to cystone.

Relative kidney weight (RKW)

The relative kidney weight (%) of normal, control and treated was shown in Table 6. In group-II Relative kidney weight were significantly (P<0.001 Vs normal) increased. The treatment with Cystone 5ml/kg in group-IV and ethanolic extract of *Acorus calamus* (EAC) at dose 500mg/kg, orally to group-III were significantly (P<0.001) decreased, when compared to group-I. The Relative kidney weight at 500mg/kg was reduced near to cystone.

Graph 5. % decrease in body weight



Graph 6. Relative Kidney weight &

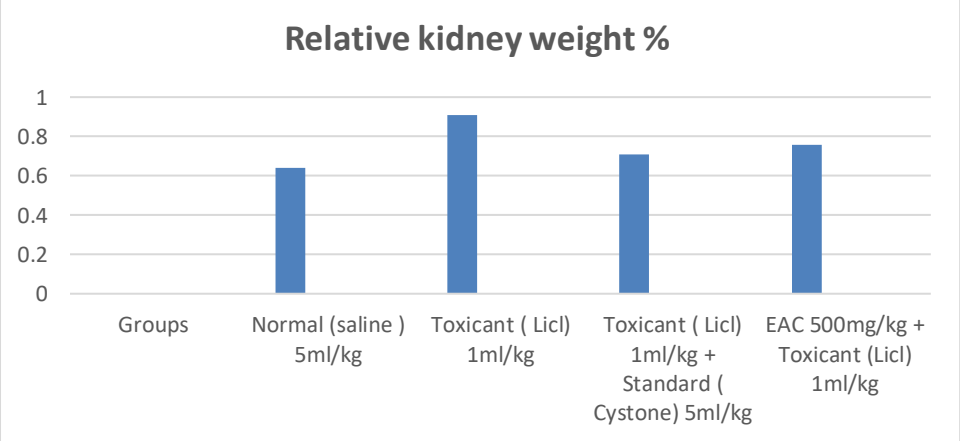


Table 3: Effect of *Acorus calamus* on serum parameters, changes in body weight and relative kidney weight against lithium chloride induced nephrotoxicity rats.

Groups	Serum parameters				Weights	
	Total protein	Creatinine	BUN	Urea	%decrease in BW	RKW
Normal	2.500±0.1200	0.3330±0.07140	7.660±0.3780	15.30±1.140	8.665±1.972	0.6399±0.0275
Toxicant (Lithium chloride 1ml/kg)	7.530±0.3160[a]	2.490±0.4280ns	43.30±0.6658[a]	43.80±2.680[b]	-6.425±1.060[b]	0.9080±0.06801[c]
Toxicant (Lithium chloride 1ml/kg) + Standard(cystone 5ml/kg)	3.320±0.3760***	0.2160±0.4771***	14.00±0.5860***	21.10±4.020***	5.411±0.5490***	0.7065±0.03525***
Extract of *Acorus calamus* 500mg/kg +Toxicant(Lithium chloride 1ml/kg)	2.980±0.3180***	0.2830±0.09450***	13.90±0.8080***	15.10±1.632***	3.956±0.3539***	0.7560±0.01645***

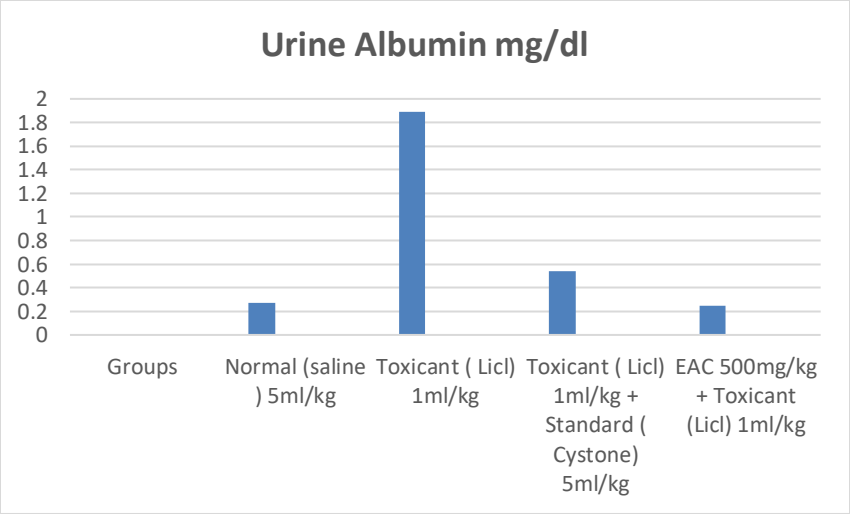
All the values are mean ± SEM, n=6 ,ns=not significant, one way analysis of variance (ANOVA) followed by dunette multiple comparison test, ***p<0.001 vs. toxicant group (lithium chloride) and [a]p<0.001, [b]p<0.01 and [c]p<0.05 vs. normal group (saline)

EFFECT OF *Acorus calamus* ON URINE PARAMETERS IN LITHIUM CHLORIDE INDUCED NEPHROTOXICITY IN RATS

Albumin

The urine albumin (mg/ml) level of normal, control and treated was shown in Table 7. The urine albumin level of normal group-I were in normal range. In group-II albumin were significantly (P<0.001 vs normal) increased. The treatment with cystone 5ml/kg in group-IV and EAC at dose 500mg/ml, orally to group-III respectively were significantly (P<0.01-0.001) decreased, when compared to control (group-II). The urine albumin level at 500mg/kg was reduced near to cystone.

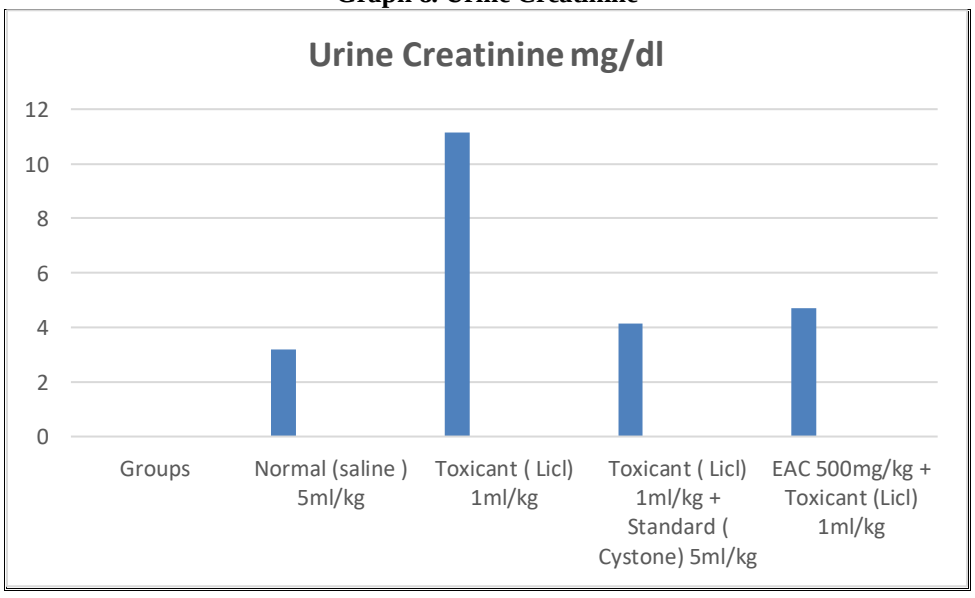
Graph 7. Urine Albumin



Creatinine

The urine creatinine (mg/dl) level of normal, control and treated was shown in Table 7.The urine creatinine level of normal group-I were in normal range. In group-II creatinine were significantly (P<0.001 vs normal) increased. The treatment with cystone 5ml/kg in group-IV and EAC at dose 500mg/ml, orally to group-III respectively were significantly (P<0.001) decreased, when compared to control (group-II). The urine albumin level at 500mg/kg was reduced near to cystone 5ml/kg.

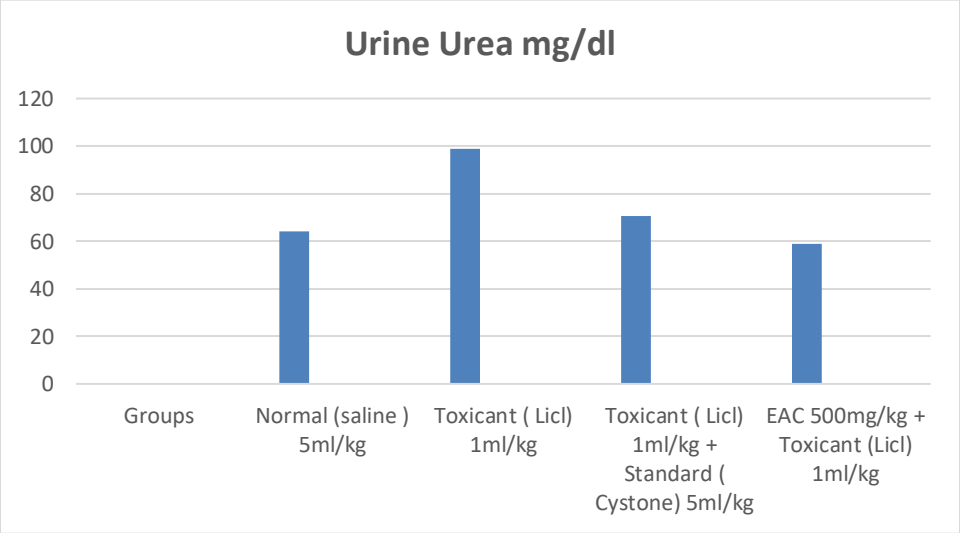
Graph 8. Urine Creatinine



Urea

The urine urea (mg/dl) level of normal, control and treated was shown in Table 7.The urine urea level of normal group-I were in normal range. In group-II urea were significantly (P<0.001 vs normal) increased. The treatment with cystone 5ml/kg in group-IV and EAC at dose 500mg/ml, orally to group-III respectively were significantly (P<0.001) decreased, when compared to control (group-II). The urine urea level at 500mg/kg was reduced near to cystone 5ml/kg.

Graph 9. Urine Urea



Uric acid

The urine uric acid (mg/dl) level of normal, control and treated was shown in Table 7.The urine uric acid level of normal group-I were in normal range. In group-II urea were significantly (P<0.001 vs normal) increased. The treatment with cystone 5ml/kg in group-IV and EAC at dose 500mg/ml, orally to group-III respectively were significantly (P<0.01-0.001) decreased, when compared to control (group-II). The urine uric acid level at 500mg/kg was reduced near to cystone 5ml/kg.

Graph 10. Urine Uric Acid

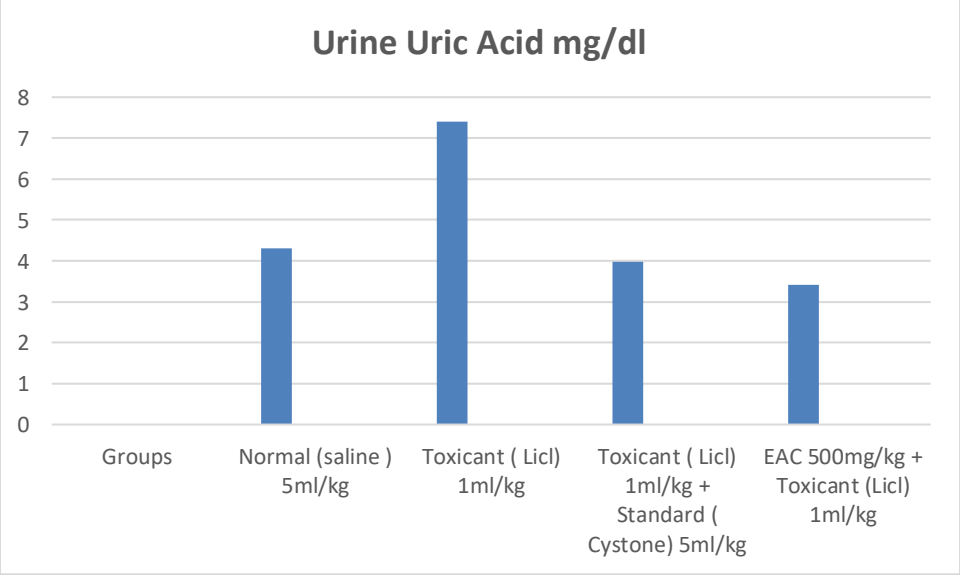


Table 4: Effect of *Acorus calamus* on urine parameters against lithium chloride induced nephrotoxicity in rats

Groups	Urine parameters			
	Albumin	Creatinine	Urea	Uric acid
Normal	0.2730±0.03200	3.180±0.1340	64.20±0.5125	4.315±0.1519
Toxicant(lithium chloride 1ml/kg)	1.890±0.1730[c]	11.16±0.5870[a]	98.92±0.3665[a]	7.400±0.1190[a]
Toxicant(lithium chloride 1ml/kg) + Standard (cystone 5ml/kg)	0.5365±0.01540***	4.150±0.03631***	70.75±0.4985***	3.970±0.06639***
Extract of *Acorus calamus* 500mg/kg + Toxicant(lithium chloride 1ml/kg)	0.2480±0.03310***	4.695±0.08900***	59.05±10.35***	3.415±0.2795***

All the values are mean ± SEM, n=6 ns=not significant, one way analysis of variance (ANOVA) followed by dunette multiple comparison test, ***p<0.001 vs toxicant group (lithium chloride) and [a]p<0.001, [c]p<0.05 vs normal group (saline)

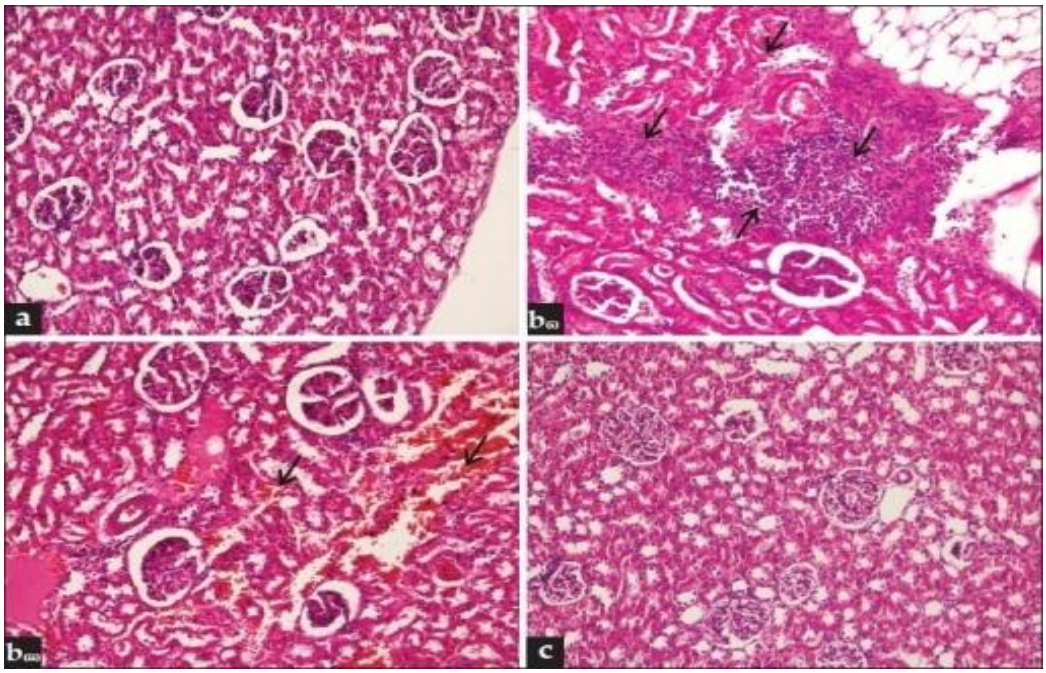


Figure: 1 Histopathology of lithium chloride induced nephrotoxicity

Renal histological analysis stained with haematoxylin and eosin dye.

a. The kidney of control rat showing normal structure
b. The kidney section of rats, with arrows showing areas of inflammation and haemolysis
c. The kidney of group III (EAC) rats showing near normal renal structure

DISCUSSION:

Nephroprotective activity

Accumulation of drugs in renal cortex leads to nephrotoxicity. Drug deposition in kidneys depends on affinity of drugs towards kidneys and on kinetics of drug trapping process.

Lithium induced nephrotoxicity

Lithium is a valuable psychotropic drug, but it has low therapeutic index. Persons taking excessive amounts of lithium either intentionally or accidently, on chronic basis or people taking medications may be accumulated with lithium. This toxicity may also

occur on acute basis. The toxic effects of lithium include ataxia, confusion, lethargy, polyuria, seizures, coma, nausea, vomiting, diarrhea and emesis. "Syndrome of Irreversible Lithium-Effected Neurotoxicity" (SILENT), is associated with episodes of acute lithium toxicity. Over dosage, usually with plasma concentrations over 1.5 mmol Li+. Lithium toxicity is preceded by sodium depletion. Uptake of sodium by the distal tubule is hazardous and is inhibited using diureticse.g.: thiazides and this should be avoided because it increases the reabsorption of lithium in proximal convoluted tubule. Plasma concentrations is more than 2.5 mmol Li+

In stabilizing mood, the specific mechanism of action of lithium is unknown. Upon lithium ingestion, decrease in norepinephrine release and increase in serotonin synthesis takes place when lithium is distributed in CNS. After decades of controversy surrounding the long-term effects of kidney, a decreased urinary concentrating ability with a confused responsiveness of distal nephron to the action of ADH (vasopressin) is demonstrable. The evidence of lithium induced nephrotoxicity in renal function is characterizedby an increase in serum creatinine, blood urea nitrogen, urine creatinine and serum urea levels. Increase in serum creatinine level is more significant than the increase in serum urea level in the earlier phase of the renal damage. Drug- induced nephrotoxicity in animals and man can be investigated by biochemical parameters such as blood urea, serum creatinine, creatinine clearance.

In the present study, the administration of 15% lithium chloride for 1 week and 7% lithium chloride for 2 weeks mixed in food as oral solution to rats caused nephrotoxicity, which is correlated with increase in creatinine, serum urea, BUN and urine creatinine levels. It was characterized by significant ($P<0.001$) elevations in the circulating levels of serum creatinine, serum urea, urine creatinine and BUN and histological features of tubulonephritis in the lithium chloride induced groups when compared with control group. However, these changes were attenuated by single daily oral dose of 500mg/kg b.w of extract of *Acorus calamus* (EAC) for respective group for 5 weeks. There was a significant ($P<0.001$) decrease in serum creatinine and urine creatinine levels in all the animals treated with extract when compared to lithium chloride induced group. Serum creatinine and urine creatinine levels were found within the normal range in groups treated with ethanolic extracts. Serum urea and BUN were observed to be significantly ($P<0.001$) decreased in EAC 500mglkg.

With long term lithium treatment, there is a reduced renal concentrating ability which is considered as a common complication. And this situation of intoxication makes the patients more susceptible to dehydration which is due to reduced fluid intake and/or increased extrarenal water or sodium loss. The reduction in renal concentrating ability is preceded by increase in plasma arginine-vasopressin concentrations which in turn is responsible for histological damage to the tubules. Nevertheless, lithium therapy should be used only for severe mood disorders. Extract of *Acorus calamus* shows neuroprotective effect against stroke and chemically induced (lithium chloride) neurodegeneration in rats. Both roots and leaves of A. calamus have shown antioxidant properties. *Acorus calamus* oil inhibits adipogenesis in 3T3-L1 cells and thus reduces lipid accumulation in fat cells.

CONCLUSSION:

Ethanolic extract of authenticated leaves of *Acorus calamus* was obtained by continuous heat extraction with soxhlet apparatus. The extracts were found to be safe up to 8000mg/kg b.w. Ethanolic extract of EAC were found to contain flavonoids, saponins, steroids and tannins. In-vivo activity were conducted with 500mg/kg b.w. *Acorus calamus* produced nephroprotective activity which was comparable to that of standard Cystone. Nephrotoxicity is one of the main side effects of lithium when it is used as antimanic drug which limits their therapeutic use. The present study reveals that ethanolic extract of *Acorus calamus* reversed nephrotoxicity induced by lithium. This indicates that EAC can be used as adjuvant with lithium compounds. This combination therapy can give therapeutic benefit without the side effect of nephrotoxicity. The phytoconstituents flavonoids, tannins, steroids and saponins present in the extract may be responsible for the anticonvulsants, antidepressant and anti-inflammatory activity.

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