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ORIGINAL ARTICLE **OPEN ACCESS**

Anti-urolithiasis and Amelioration of *Allopurinol* Induced Liver Damage with Combination of "*Embelin*" & "*Mangiferin*" in Ethylene Glycol Induced Urolithiasis in Wistar Rats

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ABSTRACT

The purpose of this study was to assess anti urolithiatic and amelioration of Allopurinol induced liver damage with combination of Embelin and Mangiferin in Albino Wistar rats by Ethylene Glycol induced Chronic Curative Study. Male Wistar Rats of weightage 150-200g were selected and divided into 5groups each comprising of 6animals each, animals were marked with picric acid according to dosage calculated based on body weight. The anti-urolithiatic and amelioration of allopurinol induced liver damage in combination with Embelin and Mangiferin was analyzed by estimation of urine, serum and kidney homogenates where in the values of calcium, phosphate, oxalate, uric acid, serum enzymes, alkaline phosphate, bilirubin, aspartate amino transferase, alanine transaminase and anti-oxidants of SOD, Catalase and GSH was calculated. Diseased toxic animals showed statistically rising electrolytes in Urine, Serum and Kidney Homogenate. Treatment with fixed dose of Embelin, Mangiferin and Allopurinol showed statistically decrease in the above parameters except urine volume and due to the administration of Allopurinol showed increased levels of alkaline phosphate, bilirubin, aspartate amino transferase, alanine transaminase and showed decreased levels in the group of animals administered with Embelin and Mangiferin. Diseased toxic animals showed statistically decrease in antioxidant activity. Treatment with fixed dose of Embelin, Mangiferin and Allopurinol exhibited significant increase in antioxidant activity. It was concluded that the Embelin and Mangiferin was having a potent anti-urolithiatic, anti-oxidant and anti-hepato toxic effect which is evident by the reduction of urine, serum and kidney homogenate levels of electrolytes, alkaline phosphate, bilirubin, aspartate aminotransferase, alanine transaminase and increased levels of SOD, catalase, and GSH was calculated.

Keywords*: Mangiferin, Embelin, Allopurinol, Antiurolithiasis, amelioration, liver damage, Albino Wistar rats.*

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INTRODUCTION

Urolithiasis is a genitourinary system pathological disease characterized by the development of calculi or stones in the bladder and kidneys. The development of calculi in the kidneys and ureters, which impede the flow of urine and cause discomfort and other symptoms, is one example. Urolithiasis can develop in the bladder or urethra in some situations [1]. Oral calcium supplements, allopurinol, thiazides, thiol sodium bicarbonate, penicillamine, aceto-hydroxamic acid, ketorolac, and other allopathic medications are used to treat kidney stones in Allopathic medicines. However, these medications have a variety of adverse effects. Dizziness, blurred vision, weakness, and other side effects may occur after using thiazide. Convulsions, lack of appetite, and weakness are all possible side effects of sodium cellulose phosphate. Rashes, fever, joint discomfort, and unusual bleeding, liver damage are all possible side effects of allopurinol [2]. The reason behind the selection of herbal medications is that Herbal medicines are inexpensive and do not necessitate surgery or hospitalization. When compared to allopathic medicines, they are recognized to have less adverse effects. Herbal medicines are also widely available and accessible to the general public [3]. The plants of present study are Embelin and Mangiferin.

Embelin is a hydroxyl benzo quinone with an alkyl substitution that was isolated from Embelia ribes Burm F., a huge scandent shrub that is widely distributed in India and belongs to the Myrsinacae family [4]. It's also known as vidanga or fake black pepper. Antibacterial, antifertility, antiprotozoal, analgesic, anti-inflammatory, antioxidant, diuretic, and other activities of Embelin have been described. Mangiferin is a polyphenolic antioxidant and glucosyl xanthone isolated from different sections of Mangifera indica, a

tropical fruiting tree in the Anacardiaceae family. Mangiferin has anti-oxidant, anti-lipid peroxidation, immunological modulation, cardiotonic, hypotensive, wound healing anti-degenerative, antiurolithiatic, and diuretic effects, among other things [5].

No scientific research or study on the anti-urolithiatic potential of Embelin and Mangiferin has been done or published as of yet. As a result, the purpose of this study is to assess the anti-urolithiatic impact of Embelin and Mangiferin, as well as the amelioration of Allopurinol-induced liver damage when Embelin and Mangiferin are used together.

MATERIAL AND METHODS

Collection of Pure Drug: Mangiferin and Embelin were procured from Vital Herbs New Delhi.

Animals:

Male Wistar rats of 150-200g weight were procured from Krupanidhi College of Pharmacy, Bangalore. They were accommodated in 12h light and dark cycle with food and water ad libitum as per CPCSEA guidelines. The protocol was approved by the Institutional Ethical Committee by the number KCP/IAEC/PCOL/55/2020 [6].

Experimental Model

The Male Wistar Albino Rats (150-200g) were weighed and randomly allocated into following groups consisting of six animals in each groups.

ANTIUROLITHIATIC STUDY

Chronic curative study

(Ethylene glycol induced urolithiasis)

Chronic Curative Study – Ethylene Glycol Induced Urolithiasis [7]:

Adult Wistar Rodents had been separated into five groups, each with six Rodents. The first group was treated normally, with standard rat food and unlimited water. Ethylene glycol (0.75%) was administered to groups II-V for 4 weeks to generate kidney calcifications. From 15th day of EG induction Group III received fixed doses of Embelin and Mangiferin respectively, group IV received Allopurinol alone at fixed dose and group V received combination of Embelin, Mangiferin and Allopurinol respectively from day 15 to day 28 along with EG in drinking water [8].

Experimental Protocol:

Group I : Normal
Group II : Positive Control
Group III : Combination of Embelin + Mangiferin (50+100mg/kg)
Group IV : Allopurinol (27mg/kg)
Group V : Combination of Embelin + Mangiferin + Allopurinol

Ethylene Glycol Induced (day 1-28)

(Dose calculations were made according to the OECD guidelines**)**

Estimations:

1. Urine: Urine volume, calcium, oxalate, phosphate, and uric acid.
2. Serum: Calcium, phosphate, oxalate and uric acid.
3. Kidney homogenate: Calcium, phosphate, oxalate and uric acid.
4. Kidney homogenate: SOD (superoxide dismutase), CAT (catalase), GSH (Glutathione)
5. Liver parameters: serum enzymes; bilirubin, alkaline phosphate, aspartate Amino-transferase, alanine-transaminase

Study Plan:

General Parameters:

Measurement of Body weight

The weight (in grams) of the animals was noted on the 14th and 28th day of Ethylene Glycol Induction and the percentage change in body weight was calculated.

Assessment of Anti-urolithiatic, antioxidant and hepato toxic Activity:

Collection Of urine:

On the 28th day, urine samples (24 hours) were taken by maintaining the animals in an individual metabolic cage. During the urine collection period, the animal had free access to drinking water. Before being refrigerated at 4°C, a drop of strong hydrochloric acid was added to the collected urine. Urine was used to determine factors such as urinary output, and urine electrolytes [9].

Collection of Serum:

Blood was collected from the retro-orbital plexus of rat under light ether anesthesia, using 1mm glass capillaries. Blood was collected in 2 ml Eppendorf tubes containing heparin. After allowing it to clot in

open for 15 min, it was centrifuged at 5000 rpm for 20 min for separation of serum. Serum obtained was stored at −20°C until further biochemical parameters estimation such as calcium, phosphate, oxalate, uric acid, and bilirubin, AST, ALT and ALP [10].

Preparation of mitochondrial supernatant:

Excess anesthetic ether was used to sacrifice the rats on the final day of the research, and the kidneys were extracted. Following weighing, a 10% kidney tissue homogenate was produced in 0.1 M Tris HCl buffer (pH-7.8) and centrifuged for 10 minutes at 4 C at 10,000 rpm. The activity of superoxide dismutase, catalase, and glutathione was measured in the supernatant.

Statistical Analysis:

Graph pad Prism version 4 software was used for statistical analysis (Graph Pad Inc, USA). ANOVA was used, followed by a Dunnett's comparison test. MEAN SEM was used to present the data. The level of confidence was set at 95%.

RESULTS

General Parameter:

When compared to the normal group, the positive control group had a huge drop in body mass ($p<0.05$). The group (3,4,5) treated with E+M+A showed significant ($^*p<0.05$) increase in body mass when compared to positive control.

$^*P<0.05$ in comparison to normal control,

$^\#P<0.05$ in comparison to toxic control

A huge drop in urine volume ($p<0.05$) was noticed in positive control when compared to the normal group. The group (3,4,5) treated with E+M+A was noticed sharp rise ($^*p<0.05$) in urine volume when compared to positive control.

A Significant rise in urine calcium, phosphate, oxalate and uric acid ($p<0.05$) was observed in positive control when compared to the normal group. The group (3,4,5) treated with E+M+A showed significant ($^*p<0.05$) decrease in urine calcium, phosphate, oxalate and uric acid when compared to positive control.

A Significant rise in serum calcium, phosphate, oxalate and uric acid ($p<0.05$) was observed in positive control when compared to the normal group. The group (3,4,5) treated with E+M+A showed significant ($^*p<0.05$) decrease in serum calcium, phosphate, oxalate and uric acid when compared to positive control.

A Significant rise in Kidney Homogenate calcium, phosphate, oxalate and uric acid ($p<0.05$) was observed in positive control when compared to the normal group. The group (3, 4, 5) treated with E+M+A showed significant ($^*p<0.05$) decrease in Kidney Homogenate calcium, phosphate, oxalate and uric acid when compared to positive control.

A Significant drop in antioxidant activity ($p<0.05$) was observed in positive control when compared to the normal group. The group (3, 4, 5) treated with E+M+A showed significant ($^*p<0.05$) increase in antioxidant activity when compared to positive control.

A Significant rise in liver parameters ($p<0.05$) was observed in allopurinol induced group (4) when compared to the normal group. The group (3) treated with E+M combination showed significant ($^*p<0.05$) decrease in liver parameters when compared to allopurinol induced group (4).

DISCUSSION

The goal of this study was to evaluate the antiurolithiatic and amelioration of allopurinol induced liver damage with combination of Embelin and Mangiferin against artificially induced kidney stones and liver damage in male wistar rats using experimental models. The anti-urolithiatic effect of Embelin, Mangiferin and Allopurinol was studied in rats by inducing urolithiasis using toxic substances like Ethylene Glycol. One of the primary variables in calculogenesis is urinary hyper saturation with regard to stone-forming components [11].

According to prior research, kidney kidney stones occurred in the young male albino rat after 2 weeks of ethylene glycol treatment, consisting primarily of calcium oxalate [12]. Hyperoxaluria, which causes increased renal retention and excessive oxalate excretion in urine, causes stone development in ethylene glycol-fed rats. Due to the obstruction of urine outflow by stones in the urinary tract, the glomerular filtration rate (GFR) decreases in urolithiasis [13]. As a result, waste products accumulate in the blood, notably nitrogenous compounds like asurea, creatinine, and uric acid.There was also an increase in lipid peroxidation and a decrease in antioxidant potential in the kidneys of rats fed a calculi-producing diet. Oxalate has been demonstrated to produce lipid peroxidation and renal tissue damage by interacting with poly unsaturated fatty acids in the cell membrane [14].

Super oxide dismutase (SOD) and catalase are free radical scavenging enzymes found in variety of cells, which converts highly reactive oxygen species to less reactive molecule thereby protect the cells from

oxidative stress. Oxidative stress due to the excessive generation of reactive radical caused a decline in the levels of catalase and SOD in the kidney tissue homogenate [15].

Positive control animals showed statistically increase in electrolytes such as calcium, phosphate, oxalate and uric acid in Urine, Serum and Kidney homogenate when compared to normal group of animals. Fixed doses of Embelin + Mangiferin + Allopurinol combination showed statistically decrease in above parameters when compared to positive control group of animals.

Positive control animals showed statistically decrease in antioxidant properties measured in kidney homogenate when compared with normal group of animals also it is evident from the results that there was significant increase in antioxidant property in the group of animals administered with the combination of Embelin + Mangiferin +Allopurinol.

The theory behind this action is uncertain, however it appears to be linked to diuresis and decreased urine concentrations of stone-forming components [16]. The ability to defend against the loss of antioxidant qualities may aid in the recovery of renal injury [17].

By utilizing the tissue homogenate it is evident that the plants of present study alsopossess in-vivo antioxidant properties along with anti-urolithiatic activity [18,19].

Treatment of Albino Wistar rats with a combination of Embelin and Mangiferin reduced hepatic cell damage by improving liver function test parameters and significantly raising antioxidant enzyme levels. The tissue repair property of Embelin [20] and Mangiferin is responsible for the restoration of liver cells [21].

Table 1. Effect of Embelin, Mangiferin and Allopurinol on Body Weight

SI. NO.	Treatment (p.o)	Body Weight Of Rats On 14th Day	Body Weight Of Rats On 28th Day
1	Normal group	185±0.846	200.666±0.494
2	Positive (EG Induced)	175±0.447*	182.6± 0.666*
3	E+M (50+100mg/Kg)	180.6±0.33#	192.3±1.145#
4	Allopurinol(27mg/Kg)	182.5±1.02#	195.6±1.085#
5	E+M+A (50+100+27mg/Kg)	183.5±2.51#	198±0.96#

Table 2: EFFECT OFEMBELIN, MANGIFERIN AND ALLOPURINOL ON URINE VOLUME

SI. NO.	Treatment (p.o)	Urine Volume (ml)
1	Normal group	6.9 ±0.25
2	Positive (EG Induced)	4.8±0.38 ***
3	E+M (50+100mg/Kg)	7.4±0.71 ###
4	Allopurinol(27mg/Kg)	9.2±0.81###
5	E+M+A (50+100+27mg/Kg)	11.3±0.74 ###

*P<0.05 in comparison to normal control,

#P<0.05 in comparison to toxic control

Table 3: Effect of Embelin, Mangiferin and Allopurinol on urine calcium

SI. NO.	Treatment (p.o)	Urine Calcium (mg/dl)
1	Normal group	6.2±0.31
2	Positive (EG Induced)	14.4±0.72 ***
3	E+M (50+100mg/Kg)	9.7± 0.56 ###
4	Allopurinol (27mg/Kg)	8.9± 0.51###
5	E+M+A (50+100+27mg/Kg)	7.6± 0.42###

Table 4: Effect of Embelin, Mangiferin and Allopurinol on urine phosphate

SI NO	Treatment (p.o)	Urine-Phosphate(mg/dl)
1	Normal group	3.6±0.29
2	Positive (EG Induced)	9.51± 0.71***
3	E+M (50+100mg/Kg)	5.51± 0.57###
4	Allopurinol (27mg/Kg)	4.6± 0.43###
5	E+M+A (50+100+27mg/Kg)	4.3± 0.36###

Table 5: Effect of Embelin, Mangiferin and Allopurinol on urine oxalate

SI NO	Treatment (p.o)	Urine-Oxalate(mg/dl)
1	**Normal group**	2.0±0.11
2	**Positive (EG Induced)**	4.5±0.12***
3	**E+M (50+100mg/Kg)**	2.23± 0.27###
4	**Allopurinol (27mg/Kg)**	2.15± 0.20###
5	**E+M+A (50+100+27mg/Kg)**	2.12±0.76###

Table 6: Effect of Embelin, Mangiferin and Allopurinol on uric acid

SI NO	Treatment (p.o)	Uric Acid(mg/dl)
1	**Normal group**	0.7±0.13
2	**Positive (EG Induced)**	5.2±0.31***
3	**E+M (50+100mg/Kg)**	2.61± 0.31###
4	**Allopurinol (27mg/Kg)**	2.3± 0.19###
5	**E+M+A (50+100+27mg/Kg)**	1.5± 0.14###

*P<0.05 in comparison to normal control, #P<0.05 in comparison to toxic control

Table 7: Effect of Embelin, Mangiferin and Allopurinol on serum calcium

SI NO	Treatment (p.o)	Serum-Calcium(mg/dl)
1	**Normal group**	3.81± 0.34
2	**Positive (EG Induced)**	13.1±0.71***
3	**E+M (50+100mg/Kg)**	5.5±0.53###
4	**Allopurinol (27mg/Kg)**	4.9± 0.47###
5	**E+M+A (50+100+27mg/Kg)**	4.6± 0.43###

Table 8: Effect of Embelin, Mangiferin and Allopurinol on serum phosphate

SI NO	Treatment (p.o)	Serum-Phosphate(mg/dl)
1	**Normal group**	5.61± 0.37
2	**Positive (EG Induced)**	14.92±0.82***
3	**E+M (50+100mg/Kg)**	8.73± 0.70 ###
4	**Allopurinol(27mg/Kg)**	7.5± 0.55 ###
5	**E+M+A (50+100+27mg/Kg)**	5.61± 0.37###

Table 9: Effect of Embelin, Mangiferin and Allopurinol on serum oxalate

SI NO	Treatment (p.o)	Serum-Oxalate(mg/dl)
1	**Normal group**	2.71±0.29
2	**Positive (EG Induced)**	5.21±0.63
3	**E+M (50+100mg/Kg)**	4.12±0.54##
4	**Allopurinol(27mg/Kg)**	4.1±0.45##
5	**E+M+A (50+100+27mg/Kg)**	2.9± 0.37###

Table 10: Effect of Embelin, Mangiferin and Allopurinol on uric acid

SI NO	Treatment (p.o)	Uric Acid (mg/dl)
1	**Normal group**	2.82± 0.07
2	**Positive (EG Induced)**	5.72± 0.30***
3	**E+M (50+100mg/Kg)**	4.6± 0.23###
4	**Allopurinol(27mg/Kg)**	4.21± 0.19###
5	**E+M+A (50+100+27mg/Kg)**	3.42 ± 0.16###

*P<0.05 in comparison to normal control, #P<0.05 in comparison to toxic control

Table 11: Effect of Embelin, Mangiferin and Allopurinol on Kidney homogenate calcium

SI NO	Treatment (p.o)	Calcium (mg/dl)
1	Normal group	4.12± 0.26
2	Positive (EG Induced)	7.32±0.3***
3	E+M (50+100mg/Kg)	6.51±0.6###
4	Allopurinol(27mg/Kg)	6.42±0.1###
5	E+M+A(50+100+27mg/Kg)	4.40±0.3###

Table 12: Effect of Embelin, Mangiferin and Allopurinol on Kidney homogenate phosphate

SI NO	Treatment (p.o)	Phosphate (mg/dl)
1	Normal group	3.54± 0.17
2	Positive (EG Induced)	7.2±0.25***
3	E+M (50+100mg/Kg)	5.2± 0.23 ###
4	Allopurinol(27mg/Kg)	4.62± 0.19 ###
5	E+M+A(50+100+27mg/Kg)	4.42± 0.18 ###

Table 13: Effect of Embelin, Mangiferin and Allopurinol on Kidney homogenate oxalate

SI NO	Treatment (p.o)	Oxalate (mg/dl)
1	Normal group	2.93± 0.22
2	Positive (EG Induced)	6.28± 0.29***
3	E+M (50+100mg/Kg)	4.41± 0.25###
4	Allopurinol(27mg/Kg)	4.3± 0.21###
5	E+M+A(50+100+27mg/Kg)	3.52± 0.17###

Table 14: Effect of Embelin, Mangiferin and Allopurinol on Kidney homogenate uric acid

SI NO	Treatment (p.o)	Uric Acid (mg/dl)
1	Normal group	2.40± 0.13
2	Positive (EG Induced)	5.4±0.28***
3	E+M (50+100mg/Kg)	3.55± 0.23###
4	Allopurinol(27mg/Kg)	3.1± 0.19###
5	E+M+A(50+100+27mg/Kg)	2.66± 0.17 ###

*P<0.05 in comparison to normal control, #P<0.05 in comparison to toxic control

Table 15: Effect of Embelin, Mangiferin and Allopurinol on SOD

SI NO	Treatment (p.o)	SOD(unit/mg protein)
1	Normal group	70.01±1.01
2	Positive (EG Induced)	11.14±0.98***
3	E+M (50+100mg/Kg)	50.12±1.02###
4	Allopurinol(27mg/Kg)	33.10±0.13###
5	E+M+A(50+100+27mg/Kg)	64.6±0.91 ###

Table 16: Effect of Embelin, Mangiferin and Allopurinol on catalase

SI NO	Treatment (p.o)	Catalase (unit/mg protein)
1	Normal group	0.62±0.02
2	Positive (EG Induced)	0.13±0.01***
3	E+M (50+100mg/Kg)	0.51±0.01 ###
4	Allopurinol(27mg/Kg)	0.42±0.01 ###
5	E+M+A(50+100+27mg/Kg)	0.53±0.01 ###

Table 17: Effect of Embelin, Mangiferin and Allopurinol on GSH

SI NO	Treatment (p.o)	GSH (unit/mg protein)
1	Normalgroup	80.12±1.61
2	Positive (EG Induced)	25.31±1.51***
3	E+M (50+100mg/Kg)	53.4±1.02 ###
4	Allopurinol(27mg/Kg)	44.61±1.12###
5	E+M+A(50+100+27mg/Kg)	55.41±3.01###

*P<0.05 in comparison to normal control, #P<0.05 in comparison to toxic control

Table 18: Effect of Embelin and Mangiferin on bilirubin

SI NO	Treatment (p.o)	Bilirubin (U/L)
1	Normal group	1.35±0.017
2	Allopurinol Induced(27mg/kg)	2.03±0.005***
3	E+M (50+100mg/Kg)	1.42±0.0070***###

Table 19: Effect of Embelin and Mangiferin on AST

SI NO	Treatment (p.o)	AST (U/L)
1	Normalgroup	67.46±0.59
2	Allopurinol Induced(27mg/kg)	130.66±1.45***
3	E+M (50+100mg/Kg)	82.5±0.76***###

Table 20: Effect of Embelin and Mangiferin on ALP

SI NO	Treatment (p.o)	ALP (U/L)
1	Normal group	96±1.06
2	Allopurinol Induced(27mg/kg)	175.3±1.70***
3	E+M (50+100mg/Kg)	128.5±0.99*** ###

Table 21: Effect of Embelin and Mangiferin on ALT

SI NO	Treatment (p.o)	ALT (U/L)
1	Normal group	32.5±0.76
2	Allopurinol Induced(27mg/kg)	94.66±1.45***
3	E+M (50+100mg/Kg)	55.16±1.47*** ###

*P<0.05 in comparison to normal control,#P<0.05 in comparison to toxic control

CONCLUSION

With the results of this study, it can be stated that Embelin and Mangiferin have a strong anti-urolithiatic and anti-oxidant effect in male wistar rats with ethylene glycol-induced urolithiasis. Also, from the above study it is evident that Embelin and Mangiferin has hepato protective action against allopurinol induced liver damage. It is worth mentioning that Embelin, Mangiferin and Allopurinol efficiently reduces the elevated levels of calcium, phosphate, oxalate and uric acid in urine, serum and kidney homogenate. And showed increased in their serum levels of anti-oxidant enzymes. In fact there is efficient reduction in the levels of AST, ALP, ALT with combination of Embelin and Mangiferin whereas Allopurinol induced group of animals showed increased levels of AST, ALP, ALT. According to the findings of this study, Embelin, Mangiferin, and Allopurinol dosing has both preventive and curative effects.

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