

## Beneficial Role of $\beta$ -Amyrin from Toothbrush Tree *Salvadora persica* in Experimental Hyperoxaluria

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Naturally occurring pentacyclic triterpenes of plant origin have been identified as possessing a wide range of pharmacological effects. The triterpene  $\beta$ -amyrin was isolated from alcoholic extract (70 % v/v ethanol) of leaves of *Salvadora persica* and the effect of oral administration of  $\beta$ -amyrin on calcium oxalate urolithiasis has been studied in male Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium, phosphate and oxalate. Increase in oxidative milieu in hyperoxaluria was also evident by increased lipid peroxidation (LPO) and decreased enzymic and non-enzymic antioxidants. Decrease in the activities of renal enzymes exemplified the damage induced by oxalate, which correlated positively with increased lipid peroxidation and increased oxalate synthesis. These abnormal biochemical aberrations were attenuated with  $\beta$ -amyrin. From the present study, it can be concluded that  $\beta$ -amyrin may serve as candidate for alleviating oxalate toxicity.

**Key Words:**  $\beta$ -Amyrin, *Salvadora persica*, Hyperoxaluria, Lipid peroxidation.

### INTRODUCTION

Urinary stones affect 10-12 % of the population in industrialized countries. Their incidence has been increasing over the past few years with the age of onset decreasing<sup>1</sup>. Although oxalate an important stone-forming constituent, is excreted mainly through the kidneys, reactive oxygen species generated during metabolism of oxalate and by oxalate itself are considered as the major contributors for the renal damage in lithogenesis. This initiates a self-perpetuating cycle ultimately leading to stone formation<sup>2</sup>. Reactive oxygen species damage the membrane and help it anchor the crystals, serving as a substratum for stone growth. It has long been recognized that antioxidants may contribute to protection against stone formation.

Structurally related triterpenes *i.e.*, lupeol (pentacyclic triterpene) have been reported to have antiurolithiatic activity in foreign body implantation techniques<sup>3</sup> and glycolate induced experimental lithiasis in rats<sup>4</sup>.  $\beta$ -Amyrin a structural analogue

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of lupeol has been reported to possess various pharmacological effects<sup>5</sup>. Recently it has been shown in our laboratory that  $\beta$ -amyrin exhibits antilithic activity in rat models.

## EXPERIMENTAL

*Salvadora persica* is a medicinal plant which possesses various biological properties<sup>6</sup>. In the present study antiurolithiatic activity was evaluated from  $\beta$ -amyrin an isolated compound from alcoholic extract of leaves of *Salvadora persica*.

Male Wistar rats (150-180 g) obtained from Madurai Medical College, Madurai were used. They were acclimatized to laboratory conditions for 1 week and maintained pellet feed. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of C.L. Baid Metha College of Pharmacy, Chennai and were in accordance with the guidelines of the committee for the purpose of control and supervision on experiments on animals (CPCSEA).

**Induction of hyperoxaluria:** Kidney stones (urolithiatic) were induced by 0.75 % v/v of ethylene glycol<sup>7</sup> in drinking water for 28 days *ad libitum*. After 28 days the animals were used for the study.

**Experimental design:** Animals were divided into six groups containing six animals in each. Group I served as control and received regular pellet diet and drinking water *ad libitum*. Ethylene glycol (0.75 % v/v) in drinking water was fed to groups II-VII for induction of renal calculi till 28th day. Group III and IV served as curative regimen (CR). Group V and VI served as preventive regimen (PR). Group III received  $\beta$ -amyrin (20 mg/kg body weight) and group IV received  $\beta$ -amyrin (40 mg/kg body weight) from 15th day till 28th day. Group V received  $\beta$ -amyrin (20 mg/kg body weight) and group VI received  $\beta$ -amyrin (40 mg/kg body weight) from 1st day till 28th day.  $\beta$ -Amyrin was dissolved in water and given once daily by oral route as solution. Results were expressed as mean  $\pm$  SEM. Differences among data were determined using one-way ANOVA followed by Tukey's multiple comparison test.

### Assessment of antiurolithiatic activity

**Collection and analysis of urine:** All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4 °C. Urine was analyzed for calcium, oxalate, phosphate, magnesium and potassium content<sup>8-10</sup>.

**Serum analysis:** After 28 days the experimental period, blood was collected from the retro-orbital under general anaesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for serum biochemical parameters such as creatinine, uric acid and urea<sup>11,12</sup>.

**Tissue homogenate analysis:** The lower abdomen was cut and both kidneys were removed from each animal. Isolated kidneys were cleared off extraneous

tissue and preserved in 10 % neutral formalin. The kidneys were dried at 80 °C in a hot air oven. A sample of 100 mg of dried kidney was boiled in 10 mL of 1 N hydrochloric acid and homogenized. The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was separated. The GSH, GPX, GAO, LDH, vitamin C, vitamin E, LPO and SOD content in kidney homogenate were determined<sup>13-20</sup>.

## RESULTS AND DISCUSSION

From the acute toxicity study, the LD<sub>50</sub> (OECD 423) cut off dose was found to be 200 mg/kg body weight for  $\beta$ -amyrin. Hence the therapeutic dose was taken as 20 mg/kg body weight.

In the present study 28 days administration of 0.75 % (v/v) ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Calcium, oxalate and phosphate excretion were grossly increased in calculi induced animals. Magnesium and potassium were decreased significantly (Table-1, group II). However, supplementation with  $\beta$ -amyrin significantly lowered the elevated levels of oxalate, calcium, phosphate and restored magnesium and potassium significantly in urine of rats with CR (curative regimen) and PR (preventive regimen) as compared to ethylene glycol treated animals.

TABLE-1  
EFFECT OF  $\beta$ -AMYRIN ON URINARY PARAMETERS

Parameter 24 h urine	Group I control	Group II Calculi induced	Curative regimen (CR)		Preventive regimen (PR)	
			Group III $\beta$ -amyrin 20 mg/kg b.wt.	Group IV $\beta$ -amyrin 40 mg/kg b.wt.	Group V $\beta$ -amyrin 20 mg/kg b.wt.	Group VI $\beta$ -amyrin 40 mg/kg b.wt.
Calcium (mg)	1.27±0.07	1.51±0.01 <sup>+a</sup>	1.28±0.06 <sup>°b</sup>	1.18±0.12 <sup>°b</sup>	1.26±0.07 <sup>°b</sup>	1.17±0.08 <sup>°b</sup>
Oxalate (mg)	0.37±0.03	3.64±0.11 <sup>+a</sup>	0.95±0.04 <sup>°b</sup>	0.85±0.06 <sup>°b</sup>	1.10±0.08 <sup>°b</sup>	0.083±0.03 <sup>°b</sup>
Potassium (mEq/L)	43±0.88	33±0.96	42±1.5 <sup>°b</sup>	46±1.7 <sup>°b</sup>	40±2.0 <sup>°b</sup>	41±1.0 <sup>°b</sup>
Phosphate (mg)	6.87±0.11	11.77±0.04 <sup>+a</sup>	9.10±0.08 <sup>°b</sup>	8.30±0.09 <sup>°b</sup>	8.50±0.06 <sup>°b</sup>	8.01±0.06 <sup>°b</sup>
Magnesium (mg)	0.92±0.01	0.50±0.01 <sup>+a</sup>	0.63±0.01 <sup>°b</sup>	0.84±0.01 <sup>°b</sup>	0.61±0.08 <sup>°b</sup>	0.79±0.02 <sup>°b</sup>

All values are expressed as mean  $\pm$  SEM for six animals in each group; (One way ANOVA Turkey's multiple comparison test); <sup>+a</sup>Statistically significant at  $p < 0.001$ . <sup>°b</sup>Statistically significant at  $p < 0.05$ . <sup>a</sup>Comparisons are made with group I; <sup>b</sup>Comparisons are made with group II.

The serum urea, uric acid and creatinine were significantly increased in calculi induced animals (Table-2, group II) indicating marked renal damage. However,  $\beta$ -amyrin treatment in curative (groups III and IV) and preventive regimen (groups V and VI) significantly ( $p < 0.001$ ) lowered the elevated serum levels of urea, uric acid and creatinine.

Table-3 delineates the activities of renal enzymes in experimental animals. The activity of GSH, GPX, SOD, vitamin C, vitamin E were significantly decreased in group II animals when compared to that of control animals. The LDH, LPO levels

TABLE-2  
EFFECT OF  $\beta$ -AMYRIN ON SERUM PARAMETERS  
IN CONTROL AND EXPERIMENTAL ANIMAL

Parameter	Group I Normal control	Group II Lithiatic control	Curative regiment (CR)		Preventive regimen (PR)	
			Group III	Group IV	Group V	Group VI
			$\beta$ -amyryn 20 mg/kg b.wt.	$\beta$ -amyryn 40 mg/kg b.wt.	$\beta$ -amyryn 20 mg/kg b.wt.	$\beta$ -amyryn 40 mg/kg b.wt.
Urea (mg/dl)	14 $\pm$ 1.8	45 $\pm$ 0.51 <sup>†a</sup>	16 $\pm$ 3.8 <sup>*b</sup>	15.02 $\pm$ 0.01 <sup>*b</sup>	17 $\pm$ 0.02 <sup>*b</sup>	14.1 $\pm$ 0.02 <sup>*b</sup>
Uric acid (mg/dl)	1.49 $\pm$ 0.07	3.64 $\pm$ 0.11 <sup>†a</sup>	2.10 $\pm$ 0.06 <sup>*b</sup>	1.11 $\pm$ 0.04 <sup>*b</sup>	2.06 $\pm$ 0.05 <sup>*b</sup>	1.94 $\pm$ 0.04 <sup>*b</sup>
Creatinine (mg/dl)	0.75 $\pm$ 0.01	0.94 $\pm$ 0.03 <sup>†a</sup>	0.92 $\pm$ 0.01 <sup>*b</sup>	0.81 $\pm$ 0.02 <sup>*b</sup>	0.91 $\pm$ 0.02 <sup>*b</sup>	0.85 $\pm$ 0.01 <sup>*b</sup>

All values are expressed as mean  $\pm$  SEM for six animals in each group; (One way ANOVA Turkey's multiple comparison test); <sup>†</sup>Statistically significant at  $p < 0.001$ ; <sup>\*</sup>Statistically significant at  $p < 0.05$ ; <sup>a</sup>Comparisons are made with group I; <sup>b</sup>Comparisons are made with group II.

TABLE-3  
EFFECT OF  $\beta$ -AMYRIN ON KIDNEY PARAMETERS OF RATS  
INTOXICATED WITH ETHYLENE GLYCOL

Parameters	Group I	Group II	Group III
	Normal control	Lithiatic control	$\beta$ -amyryn 20 mg/kg/b.wt
GSH ( $\mu$ g/mg protein)	4.84 $\pm$ 0.07	2.45 $\pm$ 0.21 <sup>†a</sup>	3.87 $\pm$ 0.21 <sup>*b</sup>
GPX ( $\mu$ g of GSH utilized/min/mg protein)	8.90 $\pm$ 0.25	5.90 $\pm$ 0.06 <sup>†a</sup>	7.20 $\pm$ 0.25 <sup>*b</sup>
LDH (Units/mg protein)	2.09 $\pm$ 0.20	5.15 $\pm$ 0.23 <sup>†a</sup>	3.74 $\pm$ 0.22 <sup>*b</sup>
Vitamin C ( $\mu$ g/mg protein)	1.56 $\pm$ 0.01	0.62 $\pm$ 0.06 <sup>†a</sup>	1.21 $\pm$ 0.01 <sup>*b</sup>
Vitamin E ( $\mu$ g/mg protein)	1.76 $\pm$ 0.08	1.02 $\pm$ 0.05 <sup>†a</sup>	1.52 $\pm$ 0.03 <sup>*b</sup>
LPO (nmol of MDA formed/mg protein)	2.01 $\pm$ 0.05	4.41 $\pm$ 0.21 <sup>†a</sup>	3.21 $\pm$ 0.16 <sup>*b</sup>
SOD (Units/mg protein)	5.60 $\pm$ 0.23	3.52 $\pm$ 0.17 <sup>†a</sup>	4.42 $\pm$ 0.25 <sup>*b</sup>
LPO (nmol of MDA formed/mg protein)	2.01 $\pm$ 0.05	4.41 $\pm$ 0.21 <sup>†a</sup>	3.21 $\pm$ 0.16 <sup>*b</sup>
SOD (Units/mg protein)	5.60 $\pm$ 0.23	3.52 $\pm$ 0.17 <sup>†a</sup>	4.42 $\pm$ 0.25 <sup>*b</sup>

  

Parameter	Group IV	Group V	Group VI
	$\beta$ -amyryn 40 mg/kg/b.wt	$\beta$ -amyryn 20 mg/kg/b.wt	$\beta$ -amyryn 40 mg/kg/b.wt
GSH ( $\mu$ g/mg protein)	–	–	–
GPX ( $\mu$ g of GSH utilized/min/mg protein)	4.24 $\pm$ 0.14 <sup>*b</sup>	3.91 $\pm$ 0.12 <sup>*b</sup>	4.45 $\pm$ 0.23 <sup>*b</sup>
LDH (Units/mg protein)	8.25 $\pm$ 0.49 <sup>*b</sup>	7.57 $\pm$ 0.36 <sup>*b</sup>	8.45 $\pm$ 0.07 <sup>*b</sup>
Vitamin C ( $\mu$ g/mg protein)	2.93 $\pm$ 0.24 <sup>*b</sup>	3.42 $\pm$ 0.19 <sup>*b</sup>	2.85 $\pm$ 0.24 <sup>*b</sup>
Vitamin E ( $\mu$ g/mg protein)	1.25 $\pm$ 0.08 <sup>*b</sup>	1.25 $\pm$ 0.03 <sup>*b</sup>	1.37 $\pm$ 0.14 <sup>*b</sup>
LPO (nmol of MDA formed/mg protein)	1.90 $\pm$ 0.12 <sup>*b</sup>	1.40 $\pm$ 0.01 <sup>*b</sup>	1.41 $\pm$ 0.03 <sup>*b</sup>
SOD (Units/mg protein)	2.81 $\pm$ 0.18 <sup>*b</sup>	3.52 $\pm$ 0.13 <sup>*b</sup>	2.92 $\pm$ 0.07 <sup>*b</sup>
LPO (nmol of MDA formed/mg protein)	2.81 $\pm$ 0.18 <sup>*b</sup>	3.52 $\pm$ 0.13 <sup>*b</sup>	2.92 $\pm$ 0.07 <sup>*b</sup>
SOD (Units/mg protein)	5.22 $\pm$ 0.09 <sup>*b</sup>	4.78 $\pm$ 0.17 <sup>*b</sup>	5.01 $\pm$ 0.11 <sup>*b</sup>

All values are expressed as mean  $\pm$  SEM for six animals in each group; (One way ANOVA Turkey's multiple comparison test); <sup>†</sup>Statistically significant at  $p < 0.001$ ; <sup>\*</sup>Statistically significant at  $p < 0.05$ ; <sup>a</sup>Comparisons are made with group I; <sup>b</sup>Comparisons are made with group II.

were significantly increased in group II animals compared to control animals. This abnormal enzymic profile was reverted to near normalcy on administration of  $\beta$ -amyryn, suggesting the membrane protective effects of the  $\beta$ -amyryn.

In the present study male rats were selected which resembles that of humans urinary system were treated with ethylene glycol (0.75 % v/v) to induce urolithiasis. Because amounts of stone deposition in female rats was significantly less<sup>21,22</sup>.

Urine parameters such as oxalate, calcium and phosphate excretion are progressively increased in calculi induced animals (Table-1, group II). Increased urinary calcium and phosphate excretion were factor favouring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth<sup>23</sup>. A low urinary magnesium and potassium content was common feature in stone formers<sup>24</sup>. However,  $\beta$ -amyrin restores the levels of the above.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this the waste products of nitrogenous substances such as urea, uric acid and creatinine got accumulated in blood<sup>25</sup>. Administration of  $\beta$ -amyrin restores the elevated level of serum urea, uric acid and creatinine.

The process of lipid peroxidation generates superoxides, aldehydes and its intermediates, which could react with essential proteins, enzymes and nucleic acids which can render tissues inactive<sup>26</sup>. In the present study, the enhanced lipid peroxidation in ethylene glycol fed rats may be due to increased promoters such as LDH and inhibitors such as GSH, GPX, Vit C, Vit E, SOD resulted in decreased antioxidant potency which leads to oxidation of membrane and tissue injury<sup>27</sup>. The present study witnessed the oxidative stress induced by ethylene glycol groups and the treatment with the  $\beta$ -amyrin restored the activities of these enzymes towards normalcy, which is indicative of antioxidant property.

In conclusion, the experimental data indicates that administration of  $\beta$ -amyrin to rats with ethylene glycol induced urolithiasis, reduced and prevented the growth of urinary stone. It is also evident from the present study that  $\beta$ -amyrin offer remarkable protection against lipid peroxidation. The mechanism underlying antiurolithic activity of  $\beta$ -amyrin is still unknown, but is apparently related to increased diuresis, lowering of urinary concentration of stone forming constituents, inhibition of oxalate induced toxic manifestations and free radical production. However, the exact role of  $\beta$ -amyrin warrants further investigations.

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