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EFFECT OF ETHANOLIC FRUIT EXTRACT OF *Prunus persica LINN.* ON MARKERS ENZYMES IN ETHYLENE GLYCOL INDUCED RATS

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ABSTRACT

The ethanolic extract of *Prunus persica* was studied for anti-urolithiatic property in ethylene glycol induced wistar rats by oral administration of extract in 150 mg/kg body weight for 28 days. The effect was measured with standard drug with the oral dose of 1mg/kg thiazide for a period of 28 days. The study estimated the effect of ethanolic fruit extract on marker enzymes in urine, liver and kidney. *Prunus persica* fruit extract showed a significant increase in AST, ALT, ACP and ALP activities in the liver and kidney when compared to ethylene glycol treated rats shows the indication of recovery proving the membrane stabilizing property and it also proves that the ethanolic fruit extract of *Prunus persica* normalized the levels of marker enzymes in tissues showing the anti-urolithiatic potential. Thus, it is concluded that *Prunus persica* has significant anti-urolithiatic activity in ethylene glycol induced wistar rats.

KEYWORDS: Urolithiasis, AST, ALT, Calcium oxalate, *Prunus persica.*

INTRODUCTION

Medicinal plants play a supreme role in the new era of modern medicine. Numerous medicinal plants and their formulations are used for various disorders. The therapeutic potential of herbs has been well recognized by various indigenous systems of medicine. The use of natural plant substances to treat and prevent illness has existed since prehistoric times and still flourishes today in many societies and cultures with many plants still in common

use. (Hemalatha K, *et al*., 2018). It is well known among different nations and it is used as safe, efficient, cultural acceptance and has fewer side effects than the synthetic drugs. The nutritional facts and minerals, the antioxidants and alkaloids compounds present in the plant and their mechanisms of the plants are based on preventing and treating diseases that were used in traditions. *(Mohammad Rahimi-Madiseh et.al., 2017).*

The medicinal plants are used widely in traditional and modern medicines such as phytopharmaceuticals, nutraceuticals, and cosmetics. WHO recognizes herbal medicine as the alternative therapy in the form of phytomedicines, herbal drugs, herbal drug preparations and herbal medicinal products *(Neeraj Tandon et.al, 2017).* The Herbal medicines are used commonly for the treatment of health issues and the use of traditional medicines may be attributable to sociocultural and community and they may contribute to disease-related problems *(Rose Kasol et.al, 2019).*

Phytochemical are naturally present in the plants and shows biologically significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. Phytochemicals is the bioactive compounds also known as secondary metabolites. There are two types of metabolites produced in plants Primary metabolites and Secondary metabolites. Primary metabolites are important for the plants regular metabolism such as growth and development. Secondary metabolites constituents are the remaining plant chemicals. (Twinkle Bansode & Dr.B.K.Salalkar, 2015).

Urolithiasis is the formation of stone anywhere in urinary tract and it is consisted as third common disease among the other diseases. It is most common in men than women. The mechanism in the stone formation in the urinary tract includes urinary super saturation, crystal nucleation, precipitation, growth, aggregation of crystals and their retention in renal tubular epithelial cells *(Salman Ahmed et.al, 2016).*

This disorder may also cause due to the oversaturation of urine with 1 or more crystal precursors, resulting in formation of crystals. The factor involved in the urolithiasis are modified with medical treatment, including the state of urinary saturation, modifiers of crystal formation, presence of multiple crystal types, and presence of bacterial infection, urinary obstruction, or foreign compounds *(Joseph W. Bartges et.al, 2015).*

The incidence has been increasing mainly due to the various reasons such as obesity and diabetes, metabolic abnormality, hypercalciuria, identifiable abnormalities (*Michelle R. Denburg et.at, 2014).* The evidences in the urolithiasis are chronic kidney disease (CKD), end-stage renal disease (ESRD), and irreversible kidney damage *(Giovanni Gambaro et. al, 2016).*

The clinical risk in the urolithiasis is pain in genitals, nausea, vomiting, hematuria, dysuria, fever, pyuria, elevated creatinine level, anuria *(Ralph C et.al, 2016).* International epidemiological Data concluded that prevalence of kidney stone disease (KSD) is increasing, and a lifetime frequency is around 14% [and a recurrence rate of 50%. It occur between the age of 40 and 60 years and now a days it also cause for the children *(Amelia Pietropaolo et.al, 2017).* It is occur all over the world and it range between in various places such as 7% - 13% in North America, 5% -9% in Europe and 1% - 5% in Asia and now also the level of urolithiasis is increasing *(Kazumi Taguchi et.al, 2017).*

The treatment of Urolithiasis is done by many ways such as Ayurveda, Siddha, Unani medicine and antiurolithiatic drugs are produced from plant. The antiurolithiatic drugs which are produced by plants such as Aerva lanata, Adiantum capillus-veneris L, Ammannia baccifera L, Bergenia ligulata, Boerhavia diffusa L, Bryophyllum pinnatum (Lam.), Crateva nurvala, Costus spiralis, Herniaria hirsuta L., Holarrhena antidysenterica, Kampo herbs, Origanum vulgare L., Phyllanthus niruri L. Zea mays L. *(Deepak M. Kasote et.al, 2017).*

Triazide is the combination of triamterene and hydrochlorothiazide. The medications called *diuretics* ("water pills"). It is used to treat the edema (fluid retention), heart failure and disorder in the liver and kidney. There are some side effects are mild or severe/temporary or permanent and it is reported in atleast 1% in the medications.

Prunus persica belongs to the family called Rosaceae and to the subfamily Amygdyloideae. It is entitled as “Aaru” and popularly known as “Peach”. Peach plays an important role in human nutrition, and dietitics. These can be used as fresh, dried or processed fruit. Peaches are nutritionally and economically essential and they are one among the most popular fruits consumed worldwide. (Ravi Kant *et al.*, 2018).

Peaches are trusted to be the "Queen" of fruits and are considered second popular after the apples. It has a mind smoothening aroma and a pleasant colour. The storage condition for

Peaches are maintained at a low temperature to reduce the quality loss at various stages such as processing, storage, transportation, sale etc. (Muhammd Naeem *et al.*, 2018).

Prunus persica is a deciduous or evergreen trees up to 10m height which is naturally distributed all over the temperate regions, basically from Asia or Southern Europe. Its bark is gray or ash in colour and is expectorant (used in cough, whooping cough, and chronic bronchitis), sedative, stomachic, demulcent, anti-scorbutic, diuretic. It has a wide variety of fruit and flesh colour and shape is unique (Ravi Kant *et al.,* 2018).

MATERIALS AND METHODS

Collection of the Fruit

Fresh *Prunus persica Linn.* (peach) fruits were collected from Ooty, The Nilgiris.

Fig 1: *Prunus persica* fruit.

Preparations of fruit extract (*Prunus persica*)

All the collected fruits that were collected from Nilgiris were cut finely and were shade dried. The shade dried fruit slices were powdered in a mixture grinder to obtain a coarse powder. This was then sieved to get fine powder of the samples. This fine powder was added to 100ml of ethanol solvent from this 1ml of extract was taken for the treatment of rats.

Selection of Animals

Healthy adult male wistar albino rats weighing about 150 to 200 grams were purchased from Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The ethical approval was got for the animals, the No: RVSCOPS/IAEC/2018/002. The rats were kept in properly numbered in large polypropylene cages with stainless steel top grill having facilities for pelleted food. Paddy husk was used as bedding material and changed twice a week. The animals were maintained in 12 hrs, light and dark cycle at 28°C ±2°C in a well-ventilated animal house under natural conditions 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.

Experimental Design

All the animals were randomly divided into four groups with six animals each, serving as normal (non-urolithiatic), urolithiatic control, urolithiatic rats treated with *Prunus persica* and standard drug (thiazide). Thiazide was given at a dose of 150µg/kg of body weight by oral administration for 28 days. The oral administration of ethanolic extract of *Prunus persica* (150 mg/b.w) was given orally for 28 days.

Table 1: Experimental design.

Group I	**Control rats** – receive normal diet
Group II	**Urolithiasis induced rats** – received 0.75% ethylene glycol in water for 28 days
Group III	**Plant drug treated rats** – urolithiasis induced rats received *Prunus persica* extract (150 mg / kg body weight) by oral administration for subsequent 28 days at a rate of 1.0 ml / rat / day.
Group IV	**Standard drug thiazide treated rats** – urolithiasis induced rats received thiazide (150 mg / kg body weight) by oral administration for subsequent 28 days at a rate of 1.0 ml/rat/day.

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages, urine was collected for 24hr. and freed from faecal contamination. Rats were provided with water but no feed. Urine collected in 50ml beaker maintained at 0^{o}C in an ice bath. The collected urine samples were centrifuged at 3000rpm for 10min. and any sediment present was discarded. It was used for further analysis.

Collection of liver and kidney samples

The experimental animals were sacrified on 29^{th} day and the liver and kidney were removed immediately, washed with ice cold saline and their weights were recorded.

Preparation of tissue homogenate

A 10% tissue homogenate was prepared by homogenizing 1.0g of chopped liver or kidney tissue in 10ml of 0.1M tris HCl homogenizing buffer at pH 7.5. The homogenate was used for assaying the enzyme activities.

Statistical analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test.

RESULTS AND DISCUSSION

Table 2: Effect of ethanolic fruit extract of *Prunus persica* Linn. on markers enzymes in urine of control and experimental rats.

GROUP	AST$^{\#\#}$	ALT$^{\#\#}$	ACP$^{\$\$}$	ALP$^{\$\$}$
I	1.98±0.08	2.52±0.08	0.54±0.04	6.55±0.07
II	5.38±0.06a*	5.79±0.07a*	0.92±0.04a*	9.800±0.07a*
III	2.52±0.06b*	3.21±0.03b*	0.73±0.03b*	7.29±0.0.0
IV	2.13±0.04c*d^{ns}	2.98±0.07c*d^{ns}	0.65±0.03c*d^{ns}	7.04±0.01c*d^{ns}

Value are expressed as mean ± SD of six animals.

Group comparison

'a' represents comparison between group II and I, 'b' represents comparison between group III and II, 'c' represents comparison between group IV and II and 'd' represents comparison between group III and IV.

Units: $^{\#\#}$µ moles of pyruvate liberated / 24 hr.urine, $^{\$\$}$µ moles of phenol liberated / 24 hr.urine.

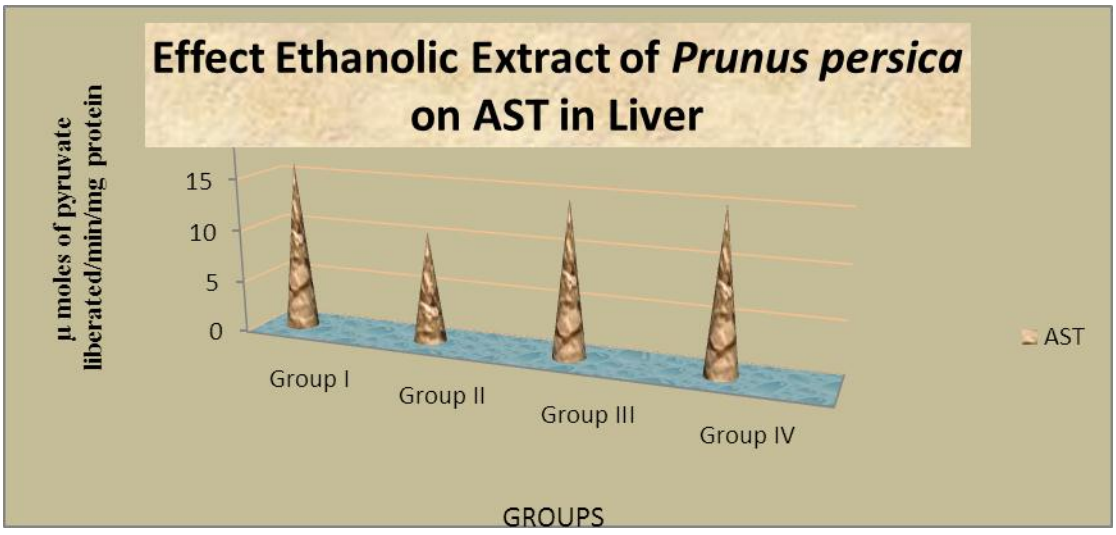

Fig 2: Effect of ethanolic fruit extract of *Prunus persica Linn.* on AST in Liver of control and experimental rats.

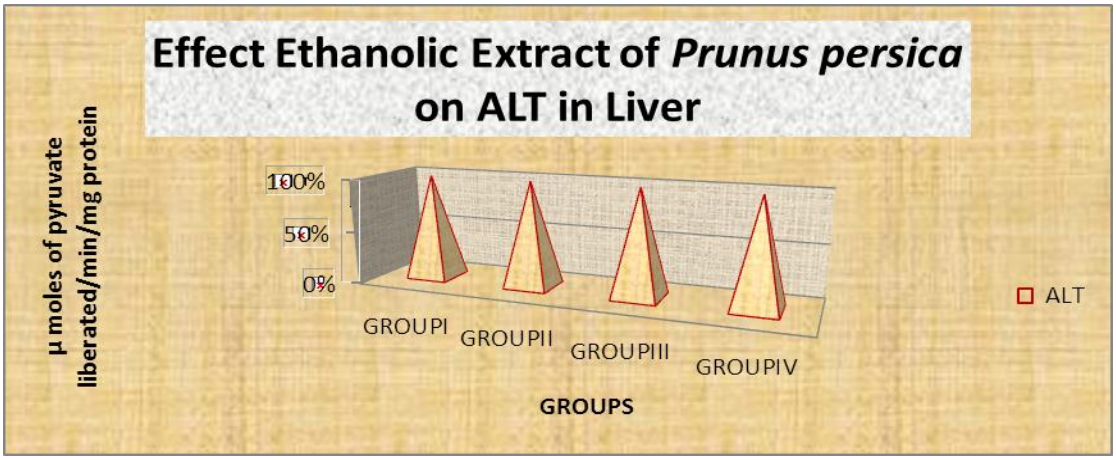

Fig 3: Effect of ethanolic fruit extract of *Prunus persica Linn.* on ALT in Liver of control and experimental rats.

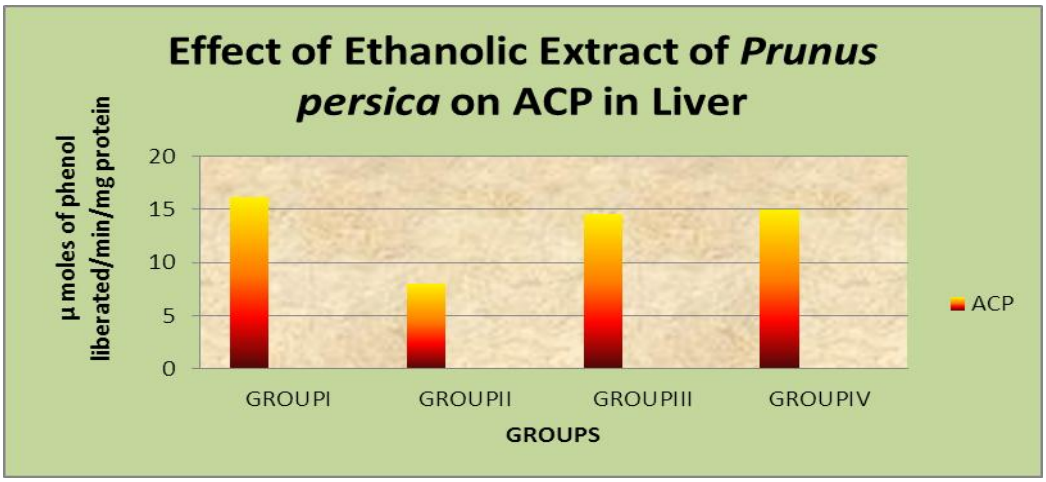

Fig 4: Effect of ethanolic fruit extract of *Prunus persica Linn*. on ACP in Liver of control and experimental rats.

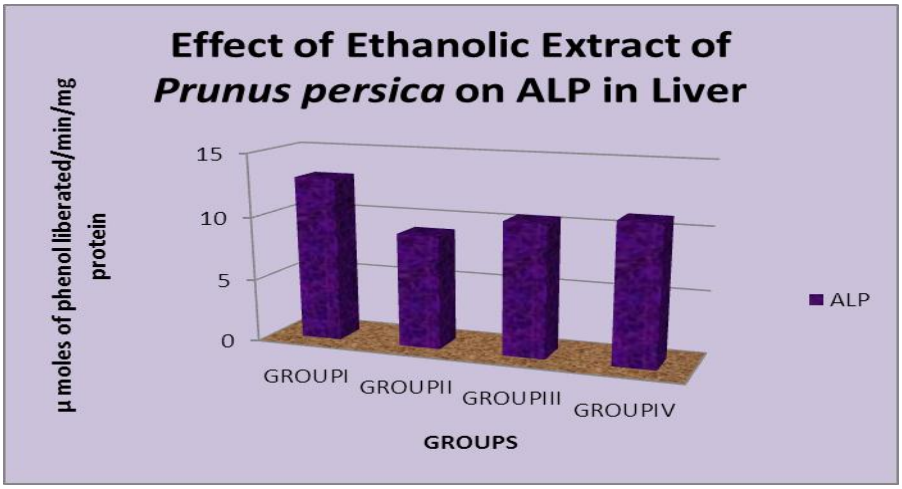

Fig 5: Effect of ethanolic fruit extract of *Prunus persica Linn.* on ALP in Liver of control and experimental rats.

Table 3: Effect of ethanolic fruit extract of *Prunus persica* Linn. on markers enzymes in Kidney of control and experimental rats.

GROUP	AST$^{\#\#}$	ALT$^{\#\#}$	ACP^{ss}	ALP^{ss}
I	11.23±0.14	12.99±0.06	11.61±0.08	6.23±0.12
II	6.99±0.08a*	9.12±0.08a*	8.14±0.08a*	3.40±0.07a*
III	10.11±0.07b*	11.91±0.04b*	10.49±0.09b*	5.80±0.06b*
IV	10.84±0.10c*d^{ns}	12.25±0.08c*d^{ns}	11.03±0.10c*d^{ns}	6.04±0.07c*d^{ns}

The values are expressed same as in Table 1.

In the present study, the rats were treated with *Prunus persica* fruit extracts (group III) after ethylene glycol induction it showed a significant ($p<0.05$) decrease in the activity of these enzymes in serum and urine when compared to group II rats. It reported that ACP and ALP levels in urine sample were increased due to administration of oxalate and their levels were maintained near normal When the standard drug treated rats (group IV) were compared with ethanolic extract of *Prunus persica* administrated group (group III), no significant difference was observed between these two groups of animals. This indicates that the activity of ethanolic extract of *prunus persica* fruit can be comparable with the standardized commercially available drug thiazide, an antiurolithiatic agent.

This result gives a supportive evidence for the antiurolithiatic activity of the ethanolic fruit extract which is similar to standard drug thiazide and the results also shows that the fruit extract has normalized the levels of marker enzymes in urine showing its membrane structure regenerating properties especially the membrane permeability.

The activities of marker enzymes (AST, ALT, ACP and ALP) in liver and kidney is evident that the activities of AST, ALT, ACP and ALP in liver and kidney homogenate were significantly decreased in ethylene glycol induced rats (group II) as compared to control rats (group I). Reduced of AST, ALT, ACP and ALP activities in hepatic and renal tissue of ethylene glycol induced rats is due to leakage of the enzymes into the general circulation from the collateral circulation.

Group III rats treated with the *Prunus persica* fruit extract showed a significant increase in AST, ALT, ACP and ALP activities in the liver and kidney when compared to ethylene glycol treated rats(group II) this is an indication of recovery proving the membrane stabilizing property of *Prunus persica.*

When thiazide treated rats (group IV) were compared with *prunus persica* fruit extract treated rats (group III), there was no significant difference between these groups of rats. This result gives a supportive evidence for the anti urolithiatic activity of ethanolic fruit extract which is similar to standard drug thiazide. It is prevalent that the ethanolic fruit extract of *Prunus persica* normalized the levels of marker enzymes in tissues showing the antiurolithiatic potential and membrane stabilizing property.

CONCLUSION

The activities of marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), acid phosphate (ACP) and alkaline phosphatase (ALT) in urine were significantly ($p<0.05$) increased. The levels were normalized in group III rats after the administration of ethanolic fruit extract of *Prunus persica.*

The levels of marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), acid phosphate (ACP) and alkaline phosphatase (ALT) were significantly ($p<0.05$) decreased in liver and kidney of urolithiatic rats (group III). Treatment with ethanolic fruit extract of *Prunus persica* restored the activity of all these enzymes in group III rats there by confirming its anti-urolithiatic potential.

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