THERAPEUTIC EFFICACY OF *RUBUS ELLIPTICUS* (SMITH) FRUITS EXTRACTS IN ACUTE ACETAMINOPHEN INDUCED NEPHROTOXICITY IN RATS

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Summary

The aim of the present investigation was to investigate the nephro-protective activity of fruits extracts of *Rubus ellipticus* (Smith) at a dose of 200 mg/kg body weight on acetaminophen (APAP) induced naphro-toxicity in male albino rats. APAP significantly increased the levels of serum creatinine, serum urea, blood urea nitrogen and kidney weight while the volume of urine reduced. The pet.ether, ethanolic and aqueous extracts of *Rubus ellipticus* fruits significantly ($p^a<0.05$, $p^b<0.01$) normalized the above said biochemical parameters increased or decreased by the oral administration of APAP (750 mg/kg body weight). Apart from these, histopathological changes also showed the protective nature of the *Rubus ellipticus* fruits extracts against APAP induced necrotic damage of renal tissues. In conclusion it was observed that all the fruits extracts of *Rubus ellipticus* conferred nephroprotective activity as evidenced by histopathological and biochemical observations against APAP induced renal damage in rats.

Key words: *Rubus ellipticus*, Naphrotoxicity, Acetaminophen (APAP).

Introduction

Acetaminophen (Paracetamol, N-Acetyl-P-Aminophenol; APAP) is a widely used analgesic and antipyretic drug that is safely employed for a wide range of treatments [1], overdose of APAP in human is fairly common and is often associated with hepatic [2,3] and renal damage [4]. Although nephrotoxicity is less common than hepatotoxicity in APAP overdose, renal tubular damage and acute renal failure can occur even in the absence of liver injury [5, 6] and can even lead to death in humans and experimental animals. Studies are going on throughout the world for the search of protective molecules that would provide maximum protection to the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body [7,8].

Rubus ellipticus Smith belonging to family Rosacea is a stout evergreen shrub grows abundantly in the forest [9, 10]. Traditionally it is used for gastralgia, wound healing, dysentery, antifertility, antimicrobial, analgesic, epilepsy, diabetes mellitus and ulcer [11, 12].

Nevertheless, the nephroprotective effects of this plant extract has not been shown in scientific research work till date. Taking this into consideration the present study is focused to evaluate the nephro-protective activity of fruits extracts of *Rubus ellipticus* against APAP induced renal-toxicity in rats.

Materials and Methods

Collection and authentification of plant materials

Fruits of *Rubus ellipticus* (smith) were obtained from local plant supplier of Etawah district India in the month of June-July 2008. The identification and authentification was done by Dr.Harish K.Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen (No.NP-102) has been submitted in college herbarium department for future reference.

Preparation of extracts

The fully ripe and shade dried fruits of *Rubus ellipticus* smith was grinded with the help of grinder. The powdered material was defatted with petroleum ether (60-80ºC) and then extracted successively with ethanol (80%) and dist. water in soxhlet apparatus. The extracts were concentrated under reduced pressure (bath temp. 50^0C). The dried extracts were then stored in an air tight container in refrigerator below 10^0C tem.

Preliminary phytochemical screening

The preliminary physiochemical investigation was carried out for the pet. ether, ethanolic, and aqueous extracts of fruits of *Rubus ellipticus* for the detection of phytoconstituents present. Test for the presence of common photochemical were carried out by standard method [13].

Determination of acute toxicity (LD_{50})

Acute toxicity test was carried out for all the extracts using OECD guide line no. 420 [14].

Experimental Animals

Wistar albino rats and swiss albino mice of both sexes were obtained from the animal house of Sir Madanlal Institute of Pharmacy, Etawah (UP) India .The animals were housed in polypropylene cages at 24±2°C and fed with commercial pellet diet and water *ad libitum.* All the animal experiments were carried out in accordance with the guidelines of CPCSEA and the study was approved by the Institutional Animal Ethics Committee (Reg.No.1225/AC/08/CPCSEA).

Chemicals and reagents

Acetaminophen was obtained from Jolly Pvt.Ltd (Etawah, UP, India). The solvent and chemicals used in the present study were of analytical grade and procured from institution central store.

Statistical analysis

Values are expressed as Mean ± SEM. The obtained data were statistically evaluated by analysis of variance (ANOVA) coupled with student t test.

Experimental Design

Acetaminophen induced nephrotoxicity in rats

The wistar albino rats of either sex (150-200 g) were divided into five groups, of six animals each.*Rubus ellipticus* fruits extracts were prepared with distilled water and acetaminophen suspension was prepared by gum tragacant (0.5%) in normal saline [15] .

Group 1: Served as normal control received only normal saline 5ml/kg, po.
Group 2: Served as + ve control received single dose of acetaminophen 750 mg/kg, po.
Group 3: Received single dose of acetaminophen 750mg/kg+ pet. ether extract 200mg/kg po for 7 days.
Group 4: Received single dose of acetaminophen750mg/kg+ ethanolic extract 200mg/kg po for 7 days.
Group 5: Received single dose of acetaminophen 750mg/kg+ Aq.extract 200mg/kg po for 7 days.

On 7th day, acetaminophen suspension was given by oral route, in a dose of 750 mg/kg body weight to all rats except the rats in group. 1 [16, 17].

Collection of blood and urine sample for biochemical analysis

Following termination of the experiment on the day 7^{th}, each animal were kept in individual metabolic cages for 24 hours for urine collection to determine the urine output. Blood samples were also collected by retro orbital puncture at the end of these 24 h. The serum was rapidly separated and processed for determination of serum urea, blood urea nitrogen (BUN) and serum creatinine using commercial available kits (Sigma). Change in body weight was recorded .Two rats per group were sacrificed by cervical decapitation and both kidneys were isolated from each rat for histological examination. The kidneys were also weighed.

Histopathological examination

Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (50–100%) alcohol, embedded in paraffin wax, cut into 4–5 µm thick sections and stained with hematoxylin–eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity such as necrosis, fatty infiltration, fibrosis, lymphocyte infiltration and blood vessel congestion etc.

Results

1. Phytochemical Investigation

The result of preliminary phytochemical investigation of different extracts is shown in Table 1.

Table 1.Preliminary phytochemical screening of *Rubus ellipticus* fruit extracts

Phytoconstituents	Pet. ether	Ethanolic	Aqueous
Carbohydrates	+	+	+
Alkaloids	+	++	++
Saponins	+	++	++
Tannins	+	++	+
Flavonoids	+	++	++
Steroids	-	+	+
Phenolic compound	+	+	+

Where: - = Absent, + = Present in low concentration, ++ = Present in high concentration.

2. Determination of acute toxicity (LD_{50})

Acute toxicity test was carried out for all the extracts using OECD guide line no. 423. No mortality or any behavioral change was found for the different extracts up to the dose of 2000 mg/kg body weight. So the dose selected for all the extracts for evaluation of nephroprotective activity was 200 mg/kg, body weight (1/10 of 2000 mg/kg body weight).

3. Biochemical tests

Effect of *Rubus ellipticus* fruits extracts on serum creatinine, serum urea, kidney weight, urine volume and blood urea nitrogen

The concentration of serum creatinine, serum urea, blood urea nitrogen and the weights of the kidneys were significant reduced ($p^{a} < 0.05$ & $p^{b} < 0.01$) in the groups [3-5] treated with different fruits extracts of *Rubus ellipticus* compared to only APAP treated group [Group 2].Table 2.

Table 2. Effect of fruits extracts of *Rubus ellipticus* on body weigh (BW), kidney weight (KW), urine volume (UV), serum creatinine (SC) serum urea (SU) and blood urea nitrogen (BUN) in acute acetaminophen nephro-toxics rats.

Groups	BW (g)	KW (g)	UV (ml)	SC(mg/ml)	SU (mg/ml)	BUN(mg/l)
Control	175±1.11	1.5 ±0.11	4.5±0.21	1.71±0.72	37.21±0.99	18.43±0.65
APAP	150± 2.10	2.1±1.01	2.3±1.00	3.11±0.71	98.12±0.98	44.21±0.65
PEE	162 ±1.23[a]	1.6±0.98[a]	3.3±0.22[b]	1.99±0.61[a]	58.00±0.53[b]	28.22±0.94[a]
EE	175±2.00[b]	1.0±0.65[b]	4.8±1.11[b]	1.73±0.92[b]	43.32 ±0.34[b]	21.31±0.8[b]
AE	168 ±0.99[a]	1.3 ±1.03[b]	3.6±1.25[b]	1.81±0.77[b]	54.41±0.51[b]	23.98±0.8[b]

Values are Mean ± SEM (n= 6), $p^{a} < 0.05$; $p^{b} < 0.01$ compared to positive control.
Where: PEE-Pet. Ether Extract, EE-Ethanolic Extract, AE-Aqueous Extract.

Effect of *Rubus ellipticus* fruits extracts on kidney histology

Protective effect were also observed in the kidney histology by concurrent oral administration of pet.ether,ethanolic and aqueous extracts of *Rubus ellipticus* fruits extracts [Fig 2-5] when compared to APAP treated group [Fig 2].

Figure 1-5. Nephroprotective effect of *Rubus ellipticus* fruits extracts on kidney histopathological observations (kidney sections stained with hematoxylin-eosin).

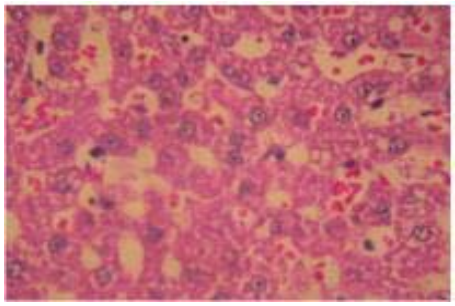

Fig 1. Normal control

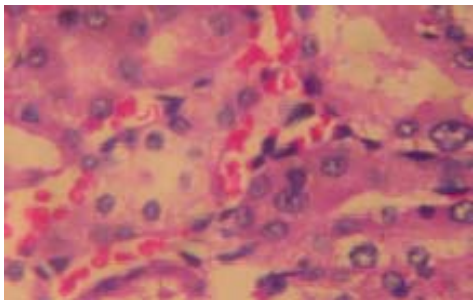

Fig 2. Acetaminophen (APAP) 750mg/kg

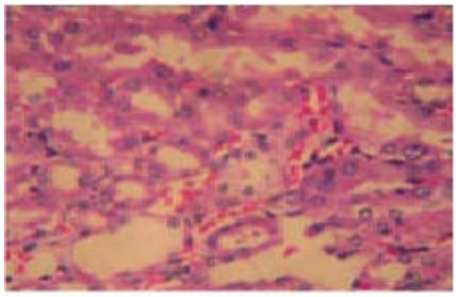

Fig 3. Pet. ether extract 200mg/kg+APAP

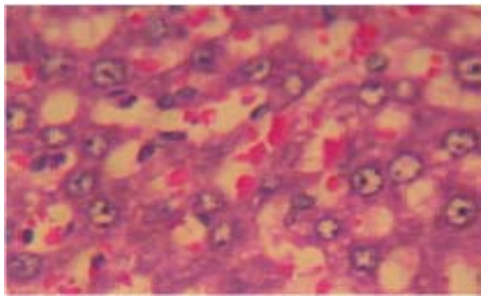

Fig 4. Ethanolic extract 200 mg/kg+APAP

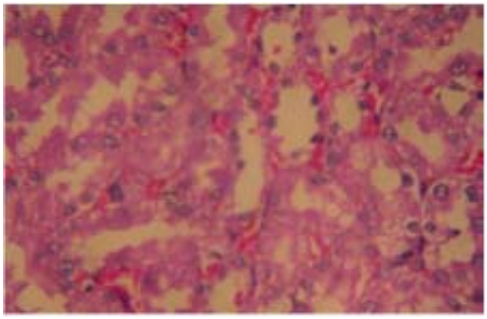

Fig 5. Aqueous extract 200mg/kg +APAP

Discussion

In recent time, the safety of acute and chronic use of acetaminophen at therapeutic dose has generated a lot of hot debates [18]. Acetaminophen over dose has been associated with significant glutathione depletion and consequent lipid peroxidation, as a result of lipid peroxidation intra cellular accumulation and covalent bonding of its highly reactive metabolite, N-acetyl-para-benzoquinone-imine (NAPQI) with hepatocytes leads to the malfunction and death of its cells [16].

Similar effect is often recorded for renal tissues. The selective renal accumulation of NSAIDS nephrotoxins including acetaminophen in animal and human is thought to result in a chain of biochemical reactions which culminate in acute or chronic nephropathies [19] in addition, acetaminophen has been reported to promote hepatocyte and renal apoptosis [20]. Acetaminophen overdose (acute or chronic) is often associated with a wide range of metabolic disorders including serum electrolytes, urea and creatinine derangements. As such, elevations in the serum concentrations of these parameters, particularly, serum urea and creatinine are considered reliable, well documented parameters for investigating drug-induced nephrotoxicity in animals and man [21].

Blood urea nitrogen is derived in the liver from proteins/amino acids, diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates (resulting in uremia) because the rate of serum urea production exceeds the rate of clearance. Other causes of uremia include high protein diet, increased catabolism due to starvation, tissue damage, sepsis or steroid treatment and absorption of amino acids and peptides from digested blood after hemorrhage into the g.i.t lumen or soft tissue.

Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatine breakdown. The plasma creatinine concentrations in normal individuals are usually affected by a number of factors such as the muscle mass, high protein diet, and catabolic state [22]. Thus, serum urea concentration is often considered a more reliable renal function predictor than serum creatinine.

In the present study, results obtained showed that acute dose acetaminophen nephrotoxicities were reliably established with 750 mg/kg/day oral acetaminophen, as evidenced by significant elevations in the blood urea nitrogen, serum urea, and serum creatinine in APAP treated control [Group.2] rats when compared to normal control rats [Group.1 and Tables.2]. Establishment of acetaminophen nephrotoxicities were also collaborated by the histological findings which showed glomeruli with loss of surrounding Bowman's capsule and varying degrees of tubular necrosis and blood vessels congestion [Fig.2] when compared to normal control rats which showed normal glomerulus with intact Bowman's capsule and tubular brush borders [Fig. 1].

However, oral treatment with the fruits extracts of *Rubus ellipticus* at a dose of 200mg/kg b.w significantly attenuated the elevated serum concentrations of these parameters [Tables 2]. The biochemical results were also confirmed by the histological findings which showed preservation of the glomeruli and the surrounding Bowman's capsule and mildly swollen tubules [Fig.3-5].

The protection offered by the extracts could have been due to the presence of any of the active principles contained in the extracts. Phytochemical screening has shown *Rubus ellipticus* to contain high concentrations of glycosides, flavonoids, alkaloids, saponins and tannins. Taking into account that flavonoids, particularly quercetin, in other nephroprotective medicinal plants have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models [23, 24] due to their potent anti-oxidant or free radicals scavenging effects [25].

In addition, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity [26, 27]. Any of these or their combination could be responsible for the observed effect.

In view of the above, one of the possible mechanisms of action of the extract could be via its antioxidant and/or free radical scavenging activities. However, this hypothesis requires validation.

In conclusion, the overall result suggests that the pet.ether, ethanolic and aqueous extracts of *Rubus ellipticus* fruits possesses nephroprotective potential and improves histological derangements associated with acute dose acetaminophen nephrotoxicity.However, the naphroprotective activity exhibited by ethanolic extract was comparatively more significant than the pet.ether and aqueous extracts of the titled plant. Although, the specific active principles were not isolated and their exact mechanisms of actions were not investigated in the present study, these could constitute an area of future studies.

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