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ACETAMINOPHEN;

REVERSAL OF ACETAMINOPHEN INDUCED NEPHROTOXIC PHENOTYPE IN MALE WISTAR RATS WITH PHOENIX DACTYLIFERA L

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ABSTRACT… Introduction: Nephrotoxicity is an important side effect of many medicine and chemotherapeutic agents. Active ingredients from natural sources have shown promising results to alleviate these side effects. **Objectives:** We aimed to investigate the effects of aqueous Date fruit extract in animal model of paracetamol induced nephrotoxicity in rats. **Study Design:** Experimental. **Setting:** Sargodha Institute of Health Sciences Sargodha. **Period:** January 2017-September 2017. **Material & Methods:** 30 rats were randomly divided into five groups treatment groups. Treatments were given daily for two weeks. Control group (Group I) is the treatment naïve one. Paracetamol (2 g/kg body weight/day) was given to group 2. Group 3 received extract of date fruit prepared in water (600 mg per kg body weight per day) for one week before paracetamol (2g per kg body weight per day) in the next week. Animals of group 4 were given paracetamol for a duration of 7 days and were then administered the extract of date palm in water. 5th group was given paracetamol (2 g per kg per day) and 600 mg extract of date fruit in water solution per kg body weight at the same time. **Results:** Renal function was recorded to be significantly altered by paracetamol toxicity and these effects were effectively reversed by the date fruit extract. **Conclusion:** The acetaminophen induced nephrotoxic changes were reversed by ductylifera in rats.

Key words: Paracetamol, Phytotherapeutics, Wistar Rats, Nephrotoxicity, Animal Model.

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INTRODUCTION

Phyto-therapeutic agents are becoming valuable treatment options in traditional as well as modern medical treatment.[1,2] The nature of natural compounds and their active and inactive contents make them much safer to use. Many of them have been reported to have no side effects.[3] Additionally, they have been pronounced as to have a protective role in many of treatment settings that have otherwise a high side effects profile. One of the most commonly encountered side effects is the nephrotoxicity. Usually it occurs when a treatment agent is metabolised via renal pathway after its metabolism. Common nephrotoxic chemicals are heavy metals, plant toxins (certain alkaloids), fungal and bacterial toxins. This toxicity may occur as a part of a treatment as well as exposure to these chemicals in house or at work place.[4] Some agents of self-harm are also known to produce nephrotoxicity.

Several chemicals have been tested for their nephroprotective potential. Traditional medicine and herbal formulations provide the best option for their determined safety status, low dose and subsequent safety profile. Many such chemicals have been tested in animal models of nephrotoxicity.[2] The common chemicals used in animal models for inducing nephrotoxicity models are either drugs that target that are cleared via renal metabolic pathway (NSAIDS and anti-tuberculous drugs) or the chemicals that are encountered on work place like alcohol or CCl_4.

Many plants with antioxidant properties have been tested for their potential role of nephrotoxicity. Among these tested plants Phoenix dactylifera L. has been known for being a significant source of energy, readily available carbohydrate content and wide availability and relatively cheap price.[5] In addition to these factors, Phoenix dactylifera L., also known as date fruit, also contains vitamin C.[6] It is a part of socially acceptable ancient staple diet for centuries and has been associated with a large number folklore in history. It also contains minute amount of nutritionally important minerals.[7] It has been used for many infectious and medical disorders in traditional medicine.[5] Due to its rich antioxidant content, it can also be a good candidate for a potentially nephroprotective role. In this study, we investigated three different effects of date fruit i.e. preventive, protective and therapeutic effects on acetaminophen (paracetamol) induced nephrotoxicity in rats.

MATERIALS AND METHODS

P. dactylifera (date palm) was purchased from local market. Seeds were removed, and it was dried under sun and then powdered by grinding. The ground powder was mixed in distilled water (@50g/Liter) and after thoroughly stirring, a sample of the solution was taken and preserved for use in the experiment. Pulverized sampling was used to check moisture, fat and total ash contents. (AOCS, 2009). Crude protein and fiber were assessed using previously published methodology.[8,9]

Experimental Protocol

This experimental study was carried out in Sargodha Institute of Health Sciences Sargodha during the period between January and September 2017. Male albino wister rats (n=30) used in this research were purchased from local vivarium. The animals were housed in shared cages (5 per cage) in temperature controlled environment (22°+/-1°C and 12-h (8AM-8PM dark) cycle. Animals were given food and water ad libitum for 2 weeks before the start of the experiments. The animals were then separated into groups of 6 animals (5 groups in total). The experimental regimes of oral intake were administered for a duration of 15 days.

Group 1 was the control group and no medication or treatment were administered. Groups 2 was administered paracetamol at the dose of 2 g/kg/day via oral gavage. Animals in group 3 were given 600 mg/kg of body weight date fruit extract for 7 days orally followed by administration of paracetamol (2 g/kg/day) via oral route. Rats in group 4 were given date fruit after a 7-day treatment with paracetamol. Group 5 rats were given paracetamol and date extract at the same time so as to monitor the protective effect of the two treatments.

At the end of the two weeks period blood samples of the animals were drawn from retro–orbital sinus under chloroform anesthesia following a fasting period of 12 hours. After centrifugation at 3000 rpm for 15 min serum was separated and stored at – 20°C. It was later used for quantification of urea (BUN), creatinine (SC) and different serum electrolytes namely sodium (Na), potassium (K), chloride (Cl) & bicarbonate (HCO_3).

Biochemical Analysis

Biochemical analyses were carried out according to previously published methodology.[8,9] The serum levels of urea were estimated by quantifying ammonia using spectrophotometer at 550mm wavelength after urea was converted to ammonia using Berthelot's reaction. Amount of creatinine in serum was measured using modified Jaffe method.[10,11] 2 mL working reagent consisting picric acid, sodium hydroxide and sodium carbonate and 0.1 ml of serum sample were mixed and kept at room temperature for 15 min. The amount was estimated by spectrophotometer at wavelength 510 mm.

As described by Schales and Schales, mercurimetric method of titration was used to estimate chloride ions concentration in serum.[12] Bicarbonate ions were estimated by van Slyke titration method in which HCl (0.1N) is added to sample and the mixture of serum sample and HCl is titrated with solution of sodium hydroxide (0.01N).[13] Flame photometric method was used to assay Na and K, as described by Mosher and Boyle.[14]

Statistical Analysis

The statistical analyses of the results were carried out using Statistical Package for the Social Sciences (SPSS) version 18.0. One-way ANOVA (analysis of variance) was used for comparison of differences by groups. Mean and standard deviation were calculated for graph presentation. and significance is taken when $P<0.05$.

RESULTS

As shown in the figure the serum concentration of urea, creatinine & sodium were significantly higher in the paracetamol treated group when compared to control group ($p<0.05$). These changes were effectively reversed in date fruit treatment groups. However, the levels of bicarbonate and chloride ion didn't change in both control and acetaminophen group. Paracetamol control (G2) and Preventive (G3) rats didn't differ in renal function parameters. However, in acetaminophen control (2) and ameliorative groups (4) the concentration of ions significantly altered ($p<0.05$). However, other parameters didn't differ significantly. However, we observed that the level of serum concentration of all kidney related parameters including urea, creatinine, Na and K decreased significantly when compared between paracetamol (G2) and the therapeutic (protective) (G5).

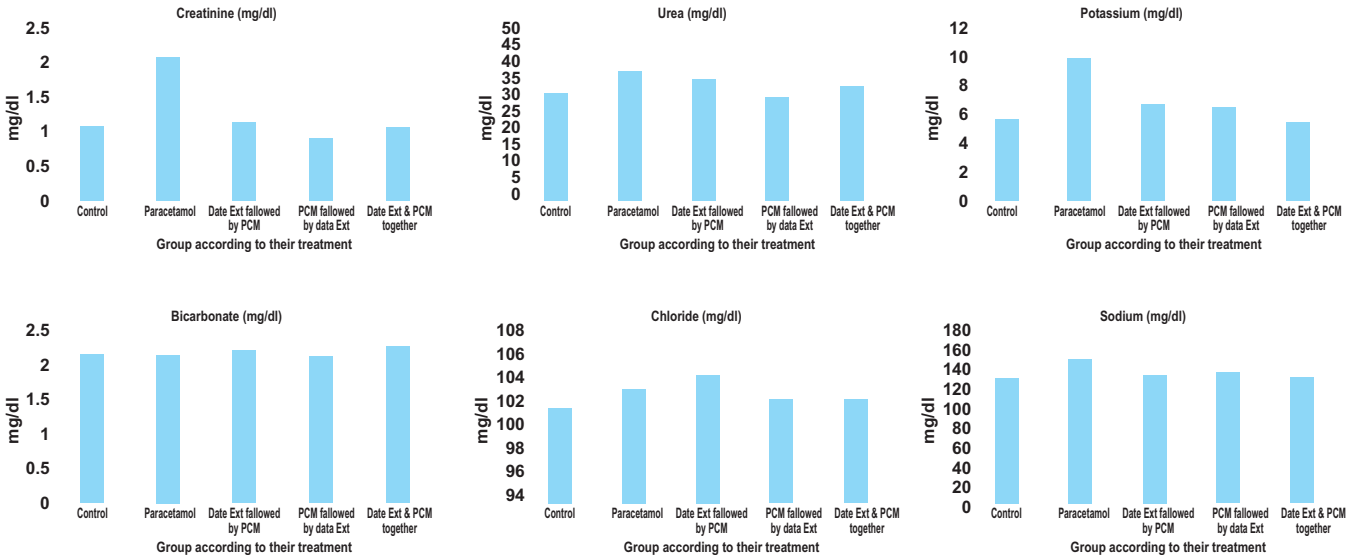

Figure-1. Statistical analysis of different parameters of nephrotoxicity between groups showing mean & standard error median. * $p<0.05$ control vs paracetamol, # $p<0.05$ paracetamol vs date extract followed by paracetamol, ¤ p,0.05 paracetamol vs paracetamol followed by date extract, § p,0.05 paracetamol vs date extract and paracetamol together

DISCUSSION

Metabolic toxic alterations are commonly found with paracetamol toxicity including electrolyte imbalance and urea and creatinine overload.[15] Paracetamol induced nephrotoxicity has already been reported in rats with major manifestations in urea and creatinine measurements.[16] The proposed mechanisms for these changes the unstopped cell death process where the antioxidant mitochondrial function is delayed.

Our results showing higher urea and creatinine values in rats exposed to paracetamol as compared to control rats are in line with the previously published literature.[17] The mechanism proposing oxidative stress responsible for the aforementioned toxic outcome in this experiment has been widely stated and accepted.[1,18] It may involve the alterations in the levels of hydrogen per oxide and reactive oxygen species (H_2O_2 and O^{2-}) which may affect both surface area & the filtration coefficient. Both these factors may contribute to bring about a decrease the filtration through glomeruli which leads to retention of urea and creatinine in the body.

Apart from urea and creatinine, we also observed electrolytes imbalance. Sodium and potassium were found to be raised in paracetamol treated group as compared to the control group. This kind of electrolyte imbalance has previously been reported in an artesunate induced model of nephrotoxicity.[19] The electrolytes concentration, especially of sodium, initiates a cascade of reactive changes stimulating the production of aldosterone that leads to augmented sodium reabsorption from tubules, reduced ADH production and brought a change in sensitivity of tubules to ADH.[20]

Potassium ion, on the other hand, plays an important role in neurotransmission. The hyperkalemia observed in paracetamol treated animals may be due to the injurious effect on sodium potassium pump that maintains concentration of K^+ in extracellular concentration. Our study, however, didn't record any alteration in bicarbonate and chloride ion which has been reported elsewhere.[19] This may be due to different causes including the doses used and duration of experiment.

The highlight of our study is the protective effect of aqueous p. dactyliferia in paracetamol induced nephrotoxicity in rats. The group of rats kept on p. dactyliferia (4) showed promising decrease in values of nephrotoxicity parameters including serum levels of creatinine, Urea, Na and K. Similar results have previously been reported using garlic oil.[21] The ameliorative effect of the aqueous extract of p. dactyleria on serum concentration of urea and creatinineis also consistent previously published literature.[22]

CONCLUSION

An animal model of Paracetamol induced nephrotoxicity in rats is effectively reversed by treatment with aqueous date palm extract.

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2	Rao Salman Aziz	Abstract, Literature review & methodology.	
3	Liaquat Ali	Performed experiments, Compile & analyze resutls.	
4	Shoaib Ahmed	Typhographical error & brief review.	
5	Maheen Rana	Spelling & grammer mistakes.	
6	Hassan Mahmood Makhdoom	Helped in finding results.	
7	Amal Shukat	Helped in performing experiments with rats arrangments.	
8	Amna Batool	Helped in performing experiments.	
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