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## CRUDE ROOT EXTRACT OF *ASPARAGUS RACEMOSUS* AMELIORATES ACETAMINOPHEN INDUCED UREMIC RATS.

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**ABSTRACT:** Now days, herbal medicines are widely used to treat, manage and cure the kidney diseases as well as reduce uremia, acute nephritis & nephritic syndrome. The present study was to evaluate the antiuremic and antioxidative effect of *Asparagus racemosus* (AR) on acetaminophen induced uremic male rats. The study was designed with 36 male albino rats which were randomly divided into 6 groups. Group I animals were provided normal food and water *ad libitum*, Group II, III, IV, V and VI received acetaminophen intraperitoneally at the dose of 500 mg/kg body weight/day for 10 days. Methanol, aqueous, hydromethanol, and hexane extract of AR at the dose of 500mg/kg body weight/day were fed orally on the 11<sup>th</sup> day and continue for next 15 days to group III, IV, V and VI respectively. After 25 days, group-II animals showed significantly increased ( $p < 0.05$ ) plasma urea, creatinine, sodium. Elevation of lipid peroxidation was noted by measuring Malondialdehyde level in both plasma and kidney tissues than Group I, III, and V. Plasma Potassium, plasma and tissue superoxide dismutase and catalase levels were significantly decreased ( $p < 0.05$ ) in animals of Group III and V as Group I. So it was concluded that the methanol and hydromethanol extract of AR conferred nephroprotective and antioxidative properties against acetaminophen induced uremia.

**INTRODUCTION:** Uremia is a condition when excess urea and other protein waste products are accumulated in the blood and shows many toxic effects that ultimately causes acute or chronic renal failure. It is a potentially fatal condition that demands immediate treatment<sup>1</sup>.

The principal derivative of chitin is Chitosan, produced by alkaline deacetylation of chitin. Treatment options for uremia includes kidney transplant and dialysis<sup>2</sup> which are very expensive, time-consuming, complicated techniques and are not free from side effects. There is great urgency for a nonconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive. In recent research findings had been showed that herbal therapy may be effective of this global problem of uremia<sup>3</sup>. Acetaminophen (APAP) is an analgesic and antipyretic drug that is widely used and safely employed for a wide range of treatments<sup>4</sup>.

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But the overdose is almost common in human and animals and is often associated with renal damage<sup>5</sup> including liver and kidney necrosis in human and animals<sup>6</sup>. 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<sup>7</sup>. A number of herbs are traditionally used in different countries in response to drug or toxin induced hepatic and renal disorders<sup>8</sup>.

*Asparagus racemosus* (AR) Wild. (family Liliaceae) locally known as Shatavari is undershrub climber with extensively tuberous root-stock. AR is traditionally used in Ayurvedic medicine from long past<sup>9</sup>. In Ayurveda, AR has been described as a *rasayana* herb and has been used extensively as an adaptogen to increase the non-specific resistance of organisms against a variety of stresses<sup>10</sup>.

AR had proved antidiarrheal<sup>11</sup>, anti-inflammatory<sup>12</sup>, neuroprotective<sup>13</sup>, Immunomodulatory<sup>14</sup>, anti-diabetic<sup>15</sup>, ulcer protecting and healing<sup>16</sup>, glucose homeostasis<sup>17</sup>, galactogogue and treatment of female reproductive system<sup>9</sup>, enhances memory<sup>18</sup>, antioxidant<sup>19</sup>, antihypercholesterolemic<sup>20</sup> etc. properties. The therapeutic components present in the root of *Asparagus racemosus* i.e. phytosterols, saponins, polyphenols, flavonoids and ascorbic acid<sup>21</sup>.

The aim of this present study was to evaluate the protective effect of aqueous, hydromethanol and methanol root extract of AR for the treatment of acetaminophen induced male albino uremic rats and which solvent extract was most effective to reduce uremia and oxidative stress against acetaminophen induced uremic and oxidative stress rats.

## MATERIALS AND METHODS:

**Drug and Chemicals:** Acetaminophen (paracetamol, *N*-acetyl *p*-aminophenol; APAP) was purchased from AshChemie India. It was administered intraperitoneally with saline water. All the chemicals used for preparation of extracts and at the time of Bio chemical tests including Urea, Creatinine, Na, K kit and Methanol, K<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, Pyragallol, Tris, TCA, TBA and other chemicals are collected from Merck Specialities Private Limited Worli. Mumbai, HiMedia

Laboratories Pvt.Ltd. Mumbai, India and Crest Biosystems Goa, India.

**Plant materials:** The root of *Asparagus racemosus* was collected from Gopali, Indian Institute of Technology, Kharagpur, Paschim Medinipur district of West Bengal. The material was identified by the taxonomist of the Botany Department at the Raja N. L. Khan Women's College, Midnapore. The voucher specimens were deposited in the Department of Botany, Raja N. L. Khan Women's College.

**Animal care and selection:** The study was conducted on 36 healthy, adult, male albino rats of Wister strain (supplied from Ghosh animals, Animal foods and animal cages supplier, Kolkata 54) having a body weight of 100 ± 20 g. They were acclimatized to laboratory conditions for two weeks prior to experimentation. The animals were grouped and housed in polyacrylic cages (38 x 23 x10 cm) three rats/cage in a temperature-controlled room (22±2°C) with 12-12 h dark-light cycles (8.00-20.00 h light, 20.00-8.00 h dark) at a humidity of 50 ± 10 %. They were provided with standard food and water ad libitum. Animal care (NIH, 1985) was provided according to the guiding principle for the care and use of animals<sup>22</sup>. This project was approved by our Institutional Animal Ethical Committee.

## Preparation of extracts of *Asparagus racemosus*

**root:** At first, 100 g of *Asparagus racemosus* root was dried at 40±1°C in incubator and the dried parts were crushed using an electric grinder and the resulting powder was then separated. Next, from these powder 25 g of each was dissolved in 250 ml of distilled water, 250 ml hydromethanol (40:60), 250 ml of methanol and 250 ml of hexane and kept in separate airtight glass jar. This mixture was incubated at 37±1°C for 72 h in an incubator cum Shaker.

After 72 h, the separate mixtures were filtered and solvent of the filtrates were completely removed by rotary vacuum evaporator. The reddish yellow coloured aqueous extract 9.542g, reddish brown hydromethanol extract 10.581g, yellowish brown of methanol extract 6.323g and the little amount of yellow coloured hexane extract 0.054g were collected (**Table 1**).

Then these extracts were dried in vacuum desiccators to obtain a dry mass, stored in a refrigerator at 4°C and used for the next 7 days of our experiments.

As per necessity, the extracts were again prepared throughout the experimental period. When needed, the four extracts were suspended in de-ionized water and used in the study<sup>23</sup>.

**Experimental design:** Thirty six healthy adult male Wister strain rats were divided into six groups with each group contains 6 rats on the basis of matching the body weights. The treatment schedule of each group was as follows:

1. **Grouping of animals:** Animals of Group I or vehicle control group were subjected to feed dry food (pellet diet) and an adequate amount of water. They received de-ionized water for 15 days prior to experimentation, followed by the next 25 days of experimental period through oral route at 8.00 a.m. through gavage. Group II, Group III, Group IV, Group V and Group VI animals were given acetaminophen at the dose of 500 mg with de-ionized water 5 mL/kg body weight/day for 10 days intraperitoneally to achieve uremia<sup>24</sup>.

The dose of acetaminophen was established in our laboratory by our previous experiment. Group II animals were then considered as uremic group and they were kept for next 15 days with normal food and water. On the 11<sup>th</sup> day, group III, IV, V and VI animals were then forcefully feed with methanol, aqueous, hydromethanol and hexane root extract of AR at the dose of 500 mg with de-ionized water 5 mL /kg body weight/day for next 15 days before giving food.

2. **Animals sacrificed and plasma and organ collection:** This experimental design was continued for 25 days. After 25 days, the animals were sacrificed and blood was collected from the aorta after which the kidneys were collected for different biochemical analysis. Blood was centrifuged at 10,000 g for 20min at 4°C and then plasma is collected.

3. **Anti-oxidant enzymes:**

- a. **Biochemical assay of catalase activity (CAT):** Catalase activity was measured biochemically. For the evaluation of CAT activity in plasma, collected blood was centrifuged and plasma fraction was separated. For kidney tissues, they were homogenized separately in 0.05 M Tris hydrochloric acid (HCl) buffer solution (pH - 7.0) at a tissue concentration of 50 mg/mL. These homogenates were centrifuged separately at 10,000 g at 4 °C for 10 min. In a spectrophotometric cuvette, 0.5 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 2.5 mL of distilled water were mixed and absorbance was determined at 240 nm. Forty microliters of tissue supernatant and plasma were separately added, and the subsequent 12 reading were noted at 30 s intervals<sup>25</sup>.

- b. **Biochemical assay of superoxide dismutase (SOD):** Kidneys were homogenized in ice-cold 100mM Tris-cocodylate buffer to give a tissue concentration of 50 mg/mL and blood centrifuged at 10,000g for 20 min at 4 °C to collect plasma. The SOD activity of these supernatants was estimated by measuring the percentage of inhibition of the pyragallol auto-oxidation by SOD. The buffer was 50 mM Tris (pH - 8.2) containing 50 mM cocodylic acid (pH - 8.2), 1 mM ethylene diamine tetra acetic acid (EDTA) and 10mM hydrochloric acid (HCl). In a spectrophotometric cuvette, 2 mL of buffer, 100 µL of 2 mM pyragallol and 10 µL of supernatant were poured and the absorbance was noted at 420 nm for 3 min. One unit of SOD was defined as the enzyme activity that inhibited the auto-oxidation of pyragallol by 50 %<sup>26</sup>.

- c. **Estimation of lipid peroxidation from the levels of malondialdehyde (MDA):** The kidneys were homogenized separately at a tissue concentration of 50 mg/mL in 0.1 M of ice-cold phosphate buffer (pH = 7.4) and the homogenates and blood samples were separately centrifuged at 10,000 g at 4 °C for 5 min. Supernatant and plasma were used for the estimation of MDA and CD. For the measurement of MDA, 0.5 mL homogenate and plasma were mixed separately with 0.5 mL normal saline and 2 mL of TBA-TCA mixture (0.392 g of TBA in 75 mL of 0.25 N HCl with 15 g of TCA, with the final volume of the mixture being made up to 100 mL with ethanol)

and, then boiled at 100°C for 10 min. The mixture was then cooled at room temperature and centrifuged at 4000 g for 10 min. The whole supernatant and plasma was transferred into a spectrophotometer cuvette and read at 535 nm. Calibration was performed by using the acid hydrolysis of 1, 1, 3, 3 tetra-methoxy propane, as a standard. The MDA present within the sample was calculated by using the extinction coefficient of  $1.56 \times 10^5$  M/cm and expressed as the unit of nM/mg of tissue or nM/mL of plasma<sup>27</sup>.

#### 4. Blood uremia profile:

a. **Biochemical estimation of blood urea:** The collected blood was centrifuged and plasma fraction was separated. Urea level of the plasma measured by commercially available standard blood urea kit (Merck, Japan), using a semi-autoanalyser (Merck, Microlab-300, Japan) as per the standard protocol for photometric determination of urea according to the urease GLDH method (kinetic UV test).

First, 10 µL of urea standard (50 mg/ 100mL) was mixed with 1000 µL of the monoreagent (composed of tris pH 7.8 120 mmol/l, 2-oxoglutarate- 7 mmol/l, ADP 0.6 mmol/l, rease 6 ku/l, glutamate dehydrogenase 1ku/l and NADH 0.25 mmol/l) and incubated for around 60 sec at 25°C, and absorbance was read at 37 °C for standardization. Then, 10 µL samples were used for the experimentation, as described before<sup>28</sup>.

b. **Biochemical estimation of blood Creatinine:** The collected blood was centrifuged and plasma fraction was separated. Creatinine level of

plasma was measured using commercially available standard blood urea kit (Merck, Japan) and a Semi-autoanalyser (Merck, Microlab-300, Japan) as per standard protocol for photometric determination of creatinine, based on Jaffe kinetic method without deproteinization.

First, 100 µL of creatinine standard (1 mg/ 100mL) was mixed with 1000 µL of the monoreagent (buffer: NaOH 313 mmol/l and picric acid 8.73 mmol/l) and incubated for around 5 min at 25°C and then absorbance was read at 37°C for standardization. Then 100 µL samples were used for analysis<sup>29</sup>.

#### 5. Electrolyte analysis:

a. **Biochemical estimation of plasma Na & K:** Sodium is precipitated as a triple salt with magnesium & uranyl acetate. The excess of uranyl ions is reacted with ferrocyanide in an acidic medium to develop a brownish colour. The intensity of the color produced is inversely proportional to the concentration of sodium in the sample. Potassium reacts with sodium tetra phenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample<sup>30</sup>.

6. **Statistical Analysis:** Analysis of variance (ANOVA) followed by a multiple two-tail 't' test with Bonferroni modification was used for statistical analysis of the collected data. Differences were considered significant when  $P < 0.05$ .

### RESULTS:

**TABLE 1: PERCENTAGE OF EXTRACT FROM WATER, HYDROMETHANOL (40:60), METHANOL AND HEXANE FROM THE ROOT OF ASPARAGUS RACEMOSUS**

Name of solvent	Amount (ml)	Amount of AR root powder (g)	Amount of extract (g)	Percentage of extract (g%)
Water	250	25	9.542	38.162
Hydromethanol (40:60 v/v)	250	25	10.581	42.32
Methanol	250	25	6.323	25.292
Hexane	250	25	0.054	0.216

**Body weight:** The body weight increased significantly ( $P < 0.05$ ) at the end of the experiment in Group I, III, IV and V compared to Group II &

VI (Table 2). In Group II animals the increase in body weight was less than the other groups due to acetaminophen induced uremia and oxidative stress.



**TABLE 2: EFFECT OF TREATMENT WITH METHANOL, AQUEOUS, HYDROMETHANOL & HEXANE ROOT EXTRACT OF AR ON BODY WEIGHT IN THE FOUR STUDY GROUPS**

Group	Initial Body Weight (g)	Final Body Weight (g)	Increases or Decreases in Body Weight (g %)
I	99.16±3.83	121±5.16 <sup>a</sup>	21.63
II	98.33±4.34	100.17±3.56 <sup>b</sup>	1.75
III	100.1±7.29	124±5.70 <sup>a</sup>	23.9
IV	105.2±6.07	129.17±6.55 <sup>a</sup>	22.62
V	98±6.733	112.5±7.47 <sup>c</sup>	14.5
VI	110.3±2.37	115±3.33 <sup>b</sup>	4.7

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ( $P < 0.05$ ). Group I: control, Group II: Uremic, Group III: Uremic + methanol extract of AR, Group IV: Uremic + aqueous extract of AR, Group V: Uremic + hydromethanol extract of AR. Group VI: Uremic + hexane extract of AR.

**Levels of Plasma Urea & Creatinine:** In this study, there is a significant ( $P < 0.05$ ) increase in the plasma urea and creatinine concentrations in the Group II animals in comparison to the Group I, Group III, IV & V. Moreover, oral administration of methanol, aqueous, hydromethanol & hexane root extract of *Asparagus racemosus* for 15 days significantly ( $P < 0.01$ ) decreased urea & creatinine level in group III and group V in comparison to the Group II, IV and VI (**Table 3**).

**TABLE 3: EFFECT OF TREATMENT OF METHANOL, AQUEOUS, HYDROMETHANOL & HEXANE EXTRACT OF ROOT OF AR, ON PLASMA UREA & CREATININE LEVEL IN THE STUDY GROUPS**

Groups	Urea (mg/dL of plasma)	Creatinine (mg/dL of plasma)
I	11.16±0.93 <sup>a</sup>	1.11±0.22 <sup>a</sup>
II	60.61±3.12 <sup>b</sup>	3.90±0.44 <sup>b</sup>
III	34.173±2.23 <sup>c</sup>	4.30±1.17 <sup>c</sup>
IV	53.91±3.94 <sup>b</sup>	2.80±0.67 <sup>c</sup>
V	31.29±2.64 <sup>c</sup>	1.94±0.47 <sup>c</sup>
VI	58.41±2.52 <sup>b</sup>	4.31±0.31 <sup>b</sup>

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ( $P < 0.05$ ). Group I: control, Group II: uremic, Group III: uremic + methanol extract of AR, Group IV: uremic + aqueous extract of AR, Group V: uremic + hydromethanol extract of AR. Group V: uremic + hexane extract of AR.

**Activities of MDA:** Plasma and kidney MDA levels was increased significantly ( $p < 0.05$ ) in group II animals in comparison to group I, III and V. On oral administration of methanol, aqueous, hydromethanol & hexane root extract of *Asparagus racemosus* after induction of uremia by acetaminophen injection for 10 days in Group III and Group V animals, the levels of MDA decreased significantly ( $P < 0.05$ ) in comparison to Group II, IV & VI (**Table 4**). So methanol and hydromethanol extract of AR significantly resettled the MDA value as to the control (group 1).

**TABLE 4: EFFECT OF TREATMENT OF METHANOL, AQUEOUS, HYDROMETHANOL & HEXANE EXTRACT OF ROOT OF AR, ON MDA ACTIVITIES IN PLASMA AND KIDNEY OF THE STUDY GROUPS**

Groups	Plasma	Kidney
	MDA (n mol/ dL of plasma)	MDA (n mol/ mg of tissue)
I	25.80±1.34 <sup>a</sup>	40.27±1.69 <sup>a</sup>
II	45.78±1.58 <sup>b</sup>	98.82±3.79 <sup>b</sup>
III	29.61±1.07 <sup>a</sup>	60.82±2.19 <sup>c</sup>
IV	42.02±2.83 <sup>b</sup>	71.36±2.54 <sup>c</sup>
V	27.61±1.07 <sup>a</sup>	66.82±2.19 <sup>c</sup>
VI	48.35±2.14 <sup>b</sup>	85.23±1.9 <sup>d</sup>

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ( $P < 0.05$ ). Group I: control, Group II: uremic, Group III: uremic + methanol extract of AR, Group IV: uremic + aqueous extract of AR, Group V: uremic + hydromethanol extract of AR, Group VI: uremic + hexane extract of AR.

**Activities of Catalase and SOD:** Uremic animals (group II) shows significant ( $p < 0.05$ ) decrease in plasma and kidney SOD and Catalase level in comparison to control (group I). When rat was treated with the methanol, aqueous, hydromethanol & hexane root extract of *Asparagus racemosus* to

group III, IV, V & VI the SOD and CAT activity was increased significantly ( $P < 0.05$ ) in Group III, IV & V when compared with group II & VI (Table

5). Also group V shows high catalase & SOD level than other groups.

**TABLE 5: EFFECT OF TREATMENT OF METHANOL, AQUEOUS, HYDROMETHANOL & HEXANE EXTRACT OF ROOT OF AR ON CATALASE AND SOD ACTIVITIES IN PLASMA AND KIDNEY OF THE STUDY GROUPS**

Groups	Plasma		Kidney	
	Catalase (m mol of H <sub>2</sub> O <sub>2</sub> consumption /dl of Plasma/min)	SOD (m mol of H <sub>2</sub> O <sub>2</sub> consumption /dl of Plasma/min)	Catalase (m mol of H <sub>2</sub> O <sub>2</sub> consumption/mg of tissue/min)	SOD (m mol of H <sub>2</sub> O <sub>2</sub> consumption/mg of tissue/min)
	I	0.911±0.04 <sup>a</sup>	0.65±0.04 <sup>a</sup>	0.87±0.05 <sup>a</sup>
II	0.343±0.02 <sup>b</sup>	0.09±0.01 <sup>b</sup>	0.22±0.03 <sup>b</sup>	0.20±0.13 <sup>b</sup>
III	1.2±0.04 <sup>c</sup>	0.34±0.07 <sup>c</sup>	1.01±0.08 <sup>c</sup>	0.92±0.14 <sup>a</sup>
IV	0.67±0.08 <sup>c</sup>	0.51±0.11 <sup>a</sup>	0.76±0.08 <sup>a</sup>	0.66±0.05 <sup>a</sup>
V	1.05±0.04 <sup>c</sup>	0.45±0.08 <sup>c</sup>	0.81±0.07 <sup>c</sup>	0.72±0.14 <sup>a</sup>
VI	0.283±0.06 <sup>b</sup>	0.19±0.05 <sup>b</sup>	0.32±0.03 <sup>b</sup>	0.16±0.03 <sup>b</sup>

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ( $P < 0.05$ ). Group I: control, Group II: uremic, Group III: uremic + methanol extract of AR, Group IV: uremic + aqueous extract of AR, Group V: uremic + hydromethanol extract of AR. Group VI: uremic + hexane extract of AR.

**Level of plasma electrolytes:** It has been revealed that there was a significant ( $P < 0.05$ ) high plasma sodium level and a significant ( $P < 0.05$ ) low plasma potassium level in Group II animals in comparison to other groups (Table 6). So due to nephrotoxicity electrolyte balance is hampered. AR treatment animals of group III and V showed a normal level of plasma sodium and potassium.

**TABLE 6: EFFECT OF TREATMENT OF METHANOL, AQUEOUS, HYDROMETHANOL AND HEXANE EXTRACT OF ROOT OF AR, ON PLASMA SODIUM & POTASSIUM LEVEL IN THE STUDY GROUPS**

Groups	Sodium (m mol/lit)	Potassium (m mol/ lit)
I	123.95±4.68 <sup>a</sup>	5.58±0.59 <sup>a</sup>
II	273.12±6.46 <sup>b</sup>	2.88±0.29 <sup>b</sup>
III	170.9±13.07 <sup>a</sup>	4.09 ±0.36 <sup>a</sup>
IV	216.74±12.0 <sup>c</sup>	5.48±0.42 <sup>a</sup>
V	140.81±8.60 <sup>a</sup>	3.68±0.25 <sup>c</sup>
VI	220.3±3.22 <sup>c</sup>	3.19±0.16 <sup>b</sup>

Data are expressed as Mean ± SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d) in a specific vertical column differ from each other significantly ( $P < 0.05$ ). Group I: control, Group II: uremic, Group III: uremic + methanol extract of AR, Group IV: uremic + aqueous extract of AR, Group V: uremic + hydromethanol extract of AR, Group VI: uremic + hexane extract of AR.

**DISCUSSION:** Acetaminophen overdose (acute or chronic) is often linked with a different type of metabolic disorders including serum electrolytes, urea and creatinine derangements. As such, elevations in the serum concentrations of these parameters, particularly, plasma urea and creatinine are considered reliable, well documented parameters for investigating drug-induced nephrotoxicity in animals and man<sup>31</sup>.

The initial step of toxicity is by significant glutathione depletion and consequent lipid peroxidation. As a consequence of lipid peroxidation, intracellular accumulation and covalent bonding of its highly reactive metabolite, N-acetyl-*para*-benzoquinone-imine (NAPQI), leading to renal injury and uremia<sup>32</sup>.

*Asparagus racemosus* roots have potent therapeutic components are phytosterols, saponins, polyphenols, flavonoids ascorbic acid, flavonoids, oligosaccharides, amino acids, sulphur and steroidal saponins<sup>20</sup> are natural antioxidants could have mainly boosted the endogenous antioxidant system in the liver and kidney of rats.

The root of *Asparagus racemosus* also responsible for increased bile production, elimination of excess cholesterol and elevation of hepatic antioxidant status in hypercholesteromic rats<sup>33</sup>. Such action of components of root of *Asparagus racemosus* could have also enhanced excretion of NAPQI and free radicals in both liver and kidneys thereby reducing toxicity of acetaminophen.

Due to acetaminophen induced uremia and oxidative stress percentage of body weight is not significantly increased in uremic group in comparison to other groups. In case of control and treatment groups body weight increased at the end of the experiment compared to their initial body weight. But in uremic rats the increased in body weight is dramatically less than the other groups. In uremia the plasma urea accumulates because the

rate of plasma urea production exceeds the rate of clearance<sup>34</sup>. Creatinine, on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown<sup>35</sup>.

Thus, plasma urea concentration is often considered a more reliable renal function predictor than plasma creatinine. In this study, acetaminophen induced nephrotoxicity showed a higher plasma urea and creatinine concentrations in the uremic animals in comparison to the methanol and hydromethanol extract of AR treated animals as control. Moreover, oral administration of hydromethanolic root extract of AR shows lower urea and creatinine level than methanol, aqueous and hexane extract of AR.

In the present study, administration of acetaminophen intraperitoneally to rats resulted in development of uremic profile & oxidative stress damage in blood & renal tissues.

Oxidative stress and lipid peroxidation are early events related to radicals generated during the hepatic metabolism of acetaminophen. It is described that a free radical mediated chain reaction damages cell membrane and MDA is a good indicator for the degree of lipid peroxidation. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity<sup>36</sup>. Previous studies have clearly demonstrated that acute acetaminophen overdose increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue<sup>37,5</sup>.

On oral administration of methanol & hydromethanol root extracts of *Asparagus racemosus* to Group III and Group V, the levels of MDA decreased significantly in comparison to uremic animals (group II) and animals of group IV & VI.

It is likely that the action of compounds present in hydromethanolic extract of *Asparagus racemosus* is mainly related to its ability to scavenge lipid peroxidation-initiating agents.

During kidney injury, superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages kidney. SOD and CAT are the most important enzymes involved in ameliorating the effects of

oxygen metabolism<sup>38</sup>. The present study also demonstrated that the administration of acetaminophen resulted in a decrease in the SOD and CAT activities, in comparison to control. It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. The increase in antioxidant enzymes might play a significant role in the mechanism of the nephroprotective effect of *Asparagus racemosus*.

In uremia, levels of sodium and potassium are one of the important parameter for electrolyte imbalance. Due to excess accumulation of waste products in body renal clearance is not properly maintained. As a result electrolytes are misbalancing. In the present study uremic animals (group II) shows imbalance of electrolytes whereas the treatment animals of Group III and V shows normal electrolyte balance in comparison to uremic group.

After administration of root extract of *Asparagus racemosus* there is a significant decrease in nephrotoxicity and oxidative stress and also maintenance of the electrolyte balance. Among four extracts of AR, hydromethanol extract shows more nephroprotective and antioxidative properties in comparison to aqueous, methanol and hexane extract against acetaminophen induced uremia.

**CONCLUSION:** We concluded that the potential use of the hydromethanol & methanol extract of *Asparagus racemosus* root could reduce renal toxicity by lowering uremic profile and protecting oxidative stress by increasing antioxidant enzymes activities on experimental animals. Also, concluded that hydromethanol extract of *Asparagus racemosus* root is more effective than aqueous, methanol & hexane extract in respect of results.

Therefore, further studies is required the mechanisms of actions of extract and phytochemical study by HPLC and NMR in future to aid the discovery of new therapeutic agents for the treatment of kidney diseases.

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