In vivo evaluation & safety profile evaluation of *Arctostaphylos uva-ursi* (L.) Spreng. extract in rabbits

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Abstract: The aim of our research work was to investigate the effects of low dose of *Arctostaphylos uva-ursi* (L.) Spreng. on rabbits. Crude extract was administered for 90 days in rabbits and hematology, biochemistry parameters and histopathology changes were analyzed. In result of it gender-based variations were observed in hematological, kidney function, liver function, cardiac enzymes and lipid profile. Urine analysis revealed same results as that of standard and control drug. No significant pathology was observed in heart, stomach, liver and kidney tissues of rabbits, treated with A.uva-ursi in a dose of 25 mg/kg/day. Our results justify the use of *A. uva-ursi* in medicine for treatment of variable pathologies.

Keywords: Bearberry, hematology, biochemical parameters, urine analysis, histopathology.

INTRODUCTION

*Arctostaphylos uva-ursi* (L.) Spreng. is a drug used for treatment of pain, diuretic, contact dermatitis, kidney stone removal and urinary tract infections (Adesunloye, 2003). A number of chemical compounds have been reported from this plant such as hydroquinone, methyl arbutin, arbutin, corilagin, allantoin, hyperoside, quercetin, kaempferol, myricetin, ellagic acid, gallic acid, malic acid, picroside, α-amyrin, ursolic acid, quinic acid, hydroquinone-O-β-D-glucoside, 6-O-galloyl arbutin, ρ-coumaric acid, syringic acid, salicylic acid etc (Bradley 1992; ESCOP 2003; British Herbal Pharmacopoeia 1996; Gruenwald et al. 2004).

Arbutin, a phenolic glycoside is the main constituent of *A. uva-ursi* that on hydrolysis is converted into hydroquinone. Both these chemicals have anti-bacterial antiseptic effect on the urinary tract (Brown, 1995). Arbutin, alone has been found to be effective in relieving pain associated with kidney stones, cystitis and nephritis as well as a diuretic (Chiej, 1984; Foster and Duke, 1990 Launert, 1981; Lust, 1983; Kavasch, 1979; Uphof, 1959; Chevallier, 1996). Ursolic acid, iso-quercetin may be used as mild diuretic (Beaux et al., 1999; Weiss, 1988). It has anti-lithic property that helps in dissolving stones in kidneys. This herb facilitates to keep the pH balance of urine from being too acidic. It actually strengthens the lining of the urinary tract and helps to get rid of any inflammation in the urinary bladder and kidneys. It has a direct relaxing effect on the bladder walls (Willard, 1992; Blumenthal et al. 1998; Siegers et al., 1997; Larsson et al. 1993).

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MATERIALS AND METHODS

*Arctostaphylos uva-ursi* (L.) Spreng. leaves were collected in 2009 from the botanical garden of the University of Karachi and identified by Prof. Dr. Mansoor Ahmad, Research Institute of Pharmaceutical Sciences, University of Karachi. A specimen voucher (FSMP-08-09) of the plant material is deposited in the herbarium of Department of Pharmacognosy, University of Karachi.

Preparation of extract

The collected fresh leaves (5 kg) of *A. uva-ursi* were air dried at 25°C and were chopped into small pieces. The chopped material was soaked in 3L ethanol. Alcoholic extract was prepared by percolation. This procedure was repeated thrice. Extract was concentrated by rotavaporator (Buchi-Rotary Evaporator, Switzerland, model # B490) at 40°C. The yield of the extract was 450gm.

Chemicals & reagents

All the chemicals and reagents used were of analytical grade and purchased from Merck (Germany).

Experimental animals

Rabbits of both sexes, weighing 1.5kg were purchased from Animal House Dow University of Health Sciences (DUHS), Karachi and kept in animal house for a period of 15 days to acclimatize. Male and female rabbits were kept in separate cages and fed with their normal diet and water. Their weights were monitored at random. The drug was administered at an interval of 24 hours for duration of 3 months. The blood of the rabbits was taken by cardiac puncture at the end of 3 months.
Animal grouping and drug dosing for hematological and biochemical evaluation

Four groups were made (male control-6 rabbits), (female control-6 rabbits), (male test (UUM)-6 rabbits) and (female control (UUF)-6 rabbits). Male and female control groups were given distilled water, while test groups UUM and UUF were given 25mg/kg A. uva-ursi. All the administration were oral. This treatment continued for 90 days. Blood (6ml) was collected by cardiac puncture with 10ml sterile syringe using 1mg/1ml EDTA as anticoagulant for the determination of blood and biochemical parameters.

Animal grouping and drug dosing for histo-pathological examination

Four groups were made namely; group I (positive control), group II (male test group without CCl₄), group III (Negative control) and group IV (male test group with CCl₄):

Group 1 (Positive control): Six animals were kept as male positive control. Water and food was provided to the animals during the entire period of experiment.

Group 2 (without CCl₄ group - male test group): Six animals were administered 25 mg/kg of test drug extract, water and food was provided to the animals during the entire period of experiment.

Group 3 (Negative control): Six animals were kept as male negative control. Water and food was provided to the animals during the entire period of experiment.

Group 4 (with CCl₄ group – male test group): Six animals were administered 25 mg/kg of Test drug extract, water and food was provided to the animals during the entire period of experiment.

The animals were sacrificed at the end of 90 days after taking out blood through cardiac puncture technique for the afore-mentioned tests. Carbon tetrachloride was injected 6 hours before taking blood for carrying out liver function test to group III & IV by cardiac puncture and sacrificing (Lucas et al., 2004). Animal studies were carried out according to Ethical Principles and Guidelines for Experiments on Animals formulated jointly by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences.

Hematological evaluation

Total erythrocyte counts were counted using a Neubar chamber under a light microscope at 40x10 magnifications. Blood samples were diluted to 200 times by Hayem’s reagent before counting. Blood hemoglobin concentration was determined using a Sahli’s hemometer. Micro Wintrobe hematocrit tubes and hematocrit centrifuge were used to determine the (PCV). Total leucocyte counts were detected using a Neubar chamber under a light microscope at 10x10 magnification after diluting blood samples to 10 times with Turk’s solution. Mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) for particular blood samples were also calculated (Burnett et al., 2006; Dacie & Lewis, 1991; McGowen et al., 1955).

Biochemical evaluation: Serum samples were obtained by centrifugation of blood at 1300xg for 15min. The Menarini Classic Chemistry Analyzer was used to determine the calcium (Ca), phosphorus (P), blood urea, creatinine, total bilirubin, total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), cholesterol, glucose, amylase, and gamma-glutamyltransferase (GGT). The globulin concentration was determined by subtracting the albumin concentration from the total protein concentration (Amadori et al., 1997; Reitman & Frankel, 1957).

Graph 1: Shows the effect of A. uva-ursi extract on the blood parameters of rabbits (male & female) in comparison with the control (male & female).

UUF = Female rabbits treated with drug; UUM = Male rabbits treated with drug

Graph 2: Shows the effect of A. uva-ursi extract on kidney function parameters of rabbit in comparison with the control.

UUF = Female rabbits treated with A. uva-ursi extract; UUM = Male rabbits treated with A. uva-ursi extract

STATISTICAL ANALYSIS

Results of the study were presented as a mean plus or minus standard error of mean (M ± SEM). Differences between control and treatment groups were analyzed by student t-test (Snedecor & Cochran, 1967).
Table 1: Complete Blood Count of Rabbits treated (UUF & UUM) with and without (Control Female & Control Male) *A. uva-ursi* extract

<table>
<thead>
<tr>
<th>Blood Parameter</th>
<th>Control Female (UUF)</th>
<th>Test Female (UUF)</th>
<th>Control Male (UUF)</th>
<th>Test Male (UUF)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>12.15±0.0836</td>
<td>9.52±0.02</td>
<td>10.05±0.0836</td>
<td>11.208±0.063</td>
<td>10.75±0.689</td>
</tr>
<tr>
<td>RBC (Erythrocyte Count)</td>
<td>5.895±0.0083</td>
<td>4.425±0.0083</td>
<td>5.485±0.0083</td>
<td>5.625±0.0083</td>
<td>3.916±0.277</td>
</tr>
<tr>
<td>Hematocrit (HCT/PVC)</td>
<td>42.835±0.0739</td>
<td>33.768±0.093</td>
<td>34.2±0.0632</td>
<td>36.85±0.083</td>
<td>38.67±1.932</td>
</tr>
<tr>
<td>MCV</td>
<td>72.416±0.0658</td>
<td>75.85±0.0836</td>
<td>62.5±0.836</td>
<td>65.53±0.11</td>
<td>89±3.183</td>
</tr>
<tr>
<td>MCH</td>
<td>20.835±0.0739</td>
<td>21.34±0.07</td>
<td>18.15±0.0836</td>
<td>19.85±0.0836</td>
<td>30.167±1.180</td>
</tr>
<tr>
<td>MCHC</td>
<td>28.783±0.0658</td>
<td>28.308±0.063</td>
<td>29.05±0.0836</td>
<td>30.325±0.068</td>
<td>32.5±0.836</td>
</tr>
<tr>
<td>Total Leucocyte Count (WBC)</td>
<td>6.05±0.0836</td>
<td>4.67±0.048</td>
<td>5.5±0.0632</td>
<td>5.35±0.0836</td>
<td>11±1.673</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>353.5±0.836</td>
<td>451.03±0.318</td>
<td>140.5±0.836</td>
<td>683.5±0.836</td>
<td>275±41.93</td>
</tr>
</tbody>
</table>

Table 2: Kidney Function Parameters of Rabbits treated (UUF & UUM) with and without (Control Female & Control Male) *A. uva-ursi* extract

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control C (female)</th>
<th>Test Animal (UUF)</th>
<th>Control C (male)</th>
<th>Test Animal (UUM)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>72.5±0.83</td>
<td>27.5±0.836</td>
<td>23.5±0.83</td>
<td>43±0.632</td>
<td>29.167±6.39</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.85±0.008</td>
<td>0.71±0.01</td>
<td>0.85±0.0083</td>
<td>0.73±0.048</td>
<td>0.8167±0.127</td>
</tr>
<tr>
<td>Calcium (serum)</td>
<td>14.59±0.063</td>
<td>15.18±0.0083</td>
<td>14.17±0.0083</td>
<td>12.475±0.0083</td>
<td>10.03±0.318</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.825±0.068</td>
<td>3.57±0.0087</td>
<td>6.195±0.0083</td>
<td>4.22±0.01</td>
<td>3.5±0.318</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.0175±0.004</td>
<td>0.03±0.0063</td>
<td>0.165±0.0083</td>
<td>0.043±0.0078</td>
<td>3.916±0.639</td>
</tr>
<tr>
<td>Total proteins</td>
<td>8±0.02</td>
<td>8.89±0.01</td>
<td>7.495±0.0083</td>
<td>7.18±0.0083</td>
<td>7.467±0.347</td>
</tr>
<tr>
<td>Albumin</td>
<td>5.83±0.013</td>
<td>4.65±0.0065</td>
<td>4.305±0.0083</td>
<td>4.19±0.01</td>
<td>4.5±0.28</td>
</tr>
<tr>
<td>Globulin</td>
<td>2.153±0.0096</td>
<td>4.22±0.01</td>
<td>3.185±0.0083</td>
<td>3.013±0.0096</td>
<td>2.35±0.146</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>2.715±0.0083</td>
<td>1.1±0.0083</td>
<td>1.35±0.016</td>
<td>1.395±0.0083</td>
<td>0.75±0.052</td>
</tr>
</tbody>
</table>

UUF = Female rabbits treated with drug; UUM = Male rabbits treated with drug

Table 3: Cardiac Enzymes Parameters of Rabbits treated (UUF & UUM) with and without (Control Female & Control Male) *A. uva-ursi* extract

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control C (female)</th>
<th>Test Animal (UUF)</th>
<th>Control C (male)</th>
<th>Test Animal (UUM)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>163.5±0.836</td>
<td>239.67±1.154</td>
<td>270.5±0.83</td>
<td>493.8±1.278</td>
<td>331.67±40.34</td>
</tr>
<tr>
<td>CPK</td>
<td>729.5±0.83</td>
<td>481.5±0.83</td>
<td>421.5±0.83</td>
<td>3052±1.166</td>
<td>90.33±23.03</td>
</tr>
<tr>
<td>CK-MB</td>
<td>852.5±0.83</td>
<td>727.5±0.836</td>
<td>194.5±0.83</td>
<td>1568.5±0.836</td>
<td>16.67±2.46</td>
</tr>
</tbody>
</table>

UUF = Female rabbits treated with drug; UUM = Male rabbits treated with drug

Table 4: Lipid Profile Parameters of Rabbits treated (UUF & UUM) with and without (Control Female & Control Male) *A. uva-ursi* extract

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control C (female)</th>
<th>Test Animal (UUF)</th>
<th>Control C (male)</th>
<th>Test Animal (UUM)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>30.5±0.83</td>
<td>83.5±0.836</td>
<td>58.5±0.83</td>
<td>49.5±0.836</td>
<td>109.16±22.24</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.5±0.83</td>
<td>159.83±1.036</td>
<td>131.5±0.83</td>
<td>34.5±0.83</td>
<td>111.67±13.68</td>
</tr>
<tr>
<td>HDL</td>
<td>12.5±0.83</td>
<td>12.5±0.836</td>
<td>6.5±0.83</td>
<td>14.5±0.836</td>
<td>19.67±3.18</td>
</tr>
<tr>
<td>LDL</td>
<td>16.5±0.83</td>
<td>48.5±0.836</td>
<td>38.5±0.83</td>
<td>36.83±7.32</td>
<td>103.33±15.14</td>
</tr>
<tr>
<td>VLDL</td>
<td>7.5±0.83</td>
<td>30.5±0.836</td>
<td>26.5±0.83</td>
<td>7±0.63</td>
<td>30±5.83</td>
</tr>
</tbody>
</table>

UUF = Female rabbits treated with drug; UUM = Male rabbits treated with drug
In vivo evaluation & safety profile evaluation of Arctostaphylos uva-ursi (L.) Spreng. extract in rabbits

Table 5: Liver Enzymes Parameters of Rabbits treated (UUF & UUM) with and without (Control Female & Control Male) A. uva-ursi extract

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Control C (Female)</th>
<th>Test Animal (UUF)</th>
<th>Control C (Male)</th>
<th>Test Animal (UUM)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT</td>
<td>26.5±0.83</td>
<td>18.67±0.96</td>
<td>42.5±0.83</td>
<td>565±0.63</td>
<td>21.83±3.11</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>0.275±0.0083</td>
<td>0.215±0.0083</td>
<td>0.265±0.0083</td>
<td>0.305±0.0083</td>
<td>1.75±0.083</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>0.021±0.005</td>
<td>0.05±0.0063</td>
<td>0.041±0.0065</td>
<td>0.095±0.0083</td>
<td>0.029±0.0008</td>
</tr>
<tr>
<td>SGPT</td>
<td>41.5±0.83</td>
<td>30.83±1.036</td>
<td>68.5±0.83</td>
<td>382.67±1.154</td>
<td>27.5±4.18</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>37.5±0.83</td>
<td>50.83±1.036</td>
<td>228.5±0.83</td>
<td>52.5±0.836</td>
<td>91.67±17.30</td>
</tr>
<tr>
<td>Gamma GT</td>
<td>6.5±0.83</td>
<td>8.5±0.836</td>
<td>9.5±0.83</td>
<td>66.5±0.836</td>
<td>29.16±6.39</td>
</tr>
</tbody>
</table>

UUF = Female rabbits treated with drug; UUM = Male rabbits treated with drug

RESULTS

Effects of A. uva-ursi on hematological and biochemical parameters are shown in tables 1-5 and graphs 1-5 respectively. Histopathology of heart, stomach, liver and kidney tissues of rabbit treated with A. uva-ursi 25mg/kg/day for 90 days are shown in fig. 1. Histopathology of heart, stomach, liver and kidney tissues of rabbits treated with A.uva-ursi 25mg/kg/day for 90 days while injected Carbon tetrachloride 6 hours prior to dissection are shown in fig. 2.

DISCUSSION

A. uva-ursi L. Spreng. is commonly called Bearberry that contains arbutin, the hydroquinone derivative as its chief active constituent and has been used since antiquity for the treatment of urinary tract infection (Small & Catling, 1999; Kosalec et al., 2008; Dykes et al., 2003). Hydroquinone exhibits anti-bacterial, astringent, disinfectant and anti-oxidant activity (Chukarina et al., 2007; Amarowicz et al., 2004). Other active constituents isolated from A. uva-ursi include phenolic acids, flavonoids, tannins, iridoids and triterpenoids (Ritch et al., 1996; Dykes et al., 2003). Pre-clinical studies revealed that A. uva-ursi potentiates the anti-inflammatory effect of corticosteroids and NSAIDs (Matsudo et al., 1990).

This research work is a new approach to recognize the effects of active constituents of A. uva-ursi extract on blood parameters of male & female rabbits (fig. 3). It is of interest to mention here that the results of A. uva-ursi extract in male rabbits’ blood are different than that of female rabbit’s blood. In male and female rabbits blood samples some parameters like leucocytes were slightly reduced whereas increase in platelet count may be due to the presence of arbutin. Overall A. uva-ursi extract showed similar effects with different values (male/female) and that is due to male and female body structures. In comparison with standard drugs and control group elevation and reduction in blood parameters were detected. These effects revealed effective utilization of A. uva-ursi in treatment of inflammatory condition like dermatitis and urinary tract infection.

The effects of A. uva-ursi extract on rabbit's kidney were observed and noted that urea (43±0.62) and albumin/globulin ratio (1.395±0.0083) were on higher side in comparison to control group of male rabbits whereas creatinine (0.73±0.48), serum calcium (12.475±0.0083), phosphorus (4.22±0.01), uric acid (0.043±0.0078), total proteins (7.185±0.0083), albumin (4.19±0.01) and globulin (3.013±0.0096) were found towards lower side in male rabbits’ blood as compared to control group. It is important to mention that there is a difference in male and female rabbits’ blood parameters. Therefore, there are differences of values in each parameter. In kidney function test (female rabbit) the effects of A. uva-ursi extract were observed raised in serum calcium (15.18±0.0083), uric acid (0.03±0.0063), total proteins (8.89±0.01), and globulin (4.22±0.01) where as the following were found towards lower side: urea (27.5±0.836), creatinine (0.71±0.01), phosphorus (3.57±0.0087), albumin (4.65±0.0065) and albumin/globulin ratio (1.1±0.0083) in comparison to control group. Our results support the utilization of A. uva-ursi in treatment of inflammation of bladder and kidneys (Willard, 1992).

A. uva-ursi extract has effects on cardiac enzyme too but male and female rabbits' showed different responses such as in case of male rabbit cardiac enzyme, that is, LDH (493.83±1.278), CPK (3052±1.166) and CK-MB (1568.5±0.836) were found raised whereas LDH (239.67±1.154) raised in comparison to control group, while CPK (481.5±0.83) and CK-MB (727.5±0.836) were on the lower side. These effects may be due to anti-oxidant potential of A. uva-ursi due to its phenolic constituents.

A. uva-ursi extract showed good response in lipid profile of both sexes. In male rabbit HDL (14.5±0.836) was found towards higher side while in the female animal it remained the same but cholesterol (83.5±0.836), triglycerides (159.83±1.036), LDL (48.5±0.836) and VLDL (30.5±0.836) were found towards higher side in comparison to female control group. Male animals showed cholesterol (49.5±0.836), triglycerides (34.5±0.836), LDL (36.83±3.72) and VLDL (7.0±0.63) at a lower side in comparison to male control group.
**Microscopic examination of heart**
Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

**Microscopic examination of stomach**
Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined.

**Microscopic examination of liver**
Sections show liver tissue with overall preserved lobular architecture. Portal tracts are within normal limits, containing portal triad and scanty fibrous tissue. No significant portal or lobular inflammation seen. No siderosis. No cholestasis. No evidence of granuloma or malignancy is seen.

**Microscopic examination of kidney**
Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows no significant pathology. Vascular structures are distributed evenly. No significant pathology is seen in any of the sections examined.

**Fig. 1**: Microscopic examination of male rabbit’s heart, stomach, liver and kidney tissues treated with *A. uva-ursi* drugs extract.

**Microscopic examination of heart**
Sections show wall of heart composed predominantly of thick myocardium consists of bundles of cardiac muscle fibers separated by fibrous band, forming syncytium. Nuclei of myocytes are centrally located. Endocardium is lined by single layer of mesothelial cells resting on a basement membrane. No significant pathology is seen in any of the sections examined.

**Microscopic examination of stomach**
Sections show wall of gastric mucosa with intact architecture. The gastric mucosa is thrown into gastric pits and folds revealing well organized glandular structures. Underlying submucosa is scanty and unremarkable. Well organized muscular layer is seen beneath, lined externally by serosa. No significant pathology is seen in any of the sections examined.

**Microscopic examination of liver**
Sections show liver tissue with overall preserved lobular architecture. Portal tracts are mildly dilated with lymphocytic infiltrate and minimal fibrosis. Sinusoidal congestion and stellate cell hyperplasia is seen. Focal macrovesicular steatosis is also of note.

**Microscopic examination of kidney**
Sections show renal tissue composed of cortex and medulla. Glomeruli are within normal limits. Tubule-interstitial compartment shows mild focal tubular atrophy and lymphocytic inflammatory infiltrate. Occasional tubular lumina show micro abscess formation. Vascular structures are distributed evenly. No evidence of granuloma or malignancy is seen.

**Fig. 2**: Microscopic examination of female rabbit’s heart, stomach, liver and kidney tissues treated with *A. uva-ursi* drugs extract.
In vivo evaluation & safety profile evaluation of Arctostaphylos uva-ursi (L.) Spreng. extract in rabbits

Corilagin
Arbutin
Methyl arbutin
Allantoin
Hyperoside
Quercetin
Kaempferol
Myricetin
Hydroquinone
Ellagic acid
Gallic acid
Malic acid
Vitamin A
Picric acid
ρ-amyrin
Ursolic acid
Quinic acid
Hydroquinone-O-β-d-glucoside
Methyl arbutin
6-O-galloyl arbutin

Continued...
ρ-coumaric acid

Syringic acid

Salicylic acid

p-hydroxybenzoic acid

Ferulic acid

Caffeic acid

Lithospermic acid

Penta-O-galloyl-β-D-Glucose

Cyanidin

Delphinidin

Quercitrin

Isoquercitrin

Myricitrin

Ursolic acid

Uvaol

β-amyrin

Arbutoside

**Fig. 3**: Reported chemical structures present in *A. uva-ursi* L.
It is also interesting to note that in liver enzyme; alkaline phosphatase (52.5±0.836) was lowered while SGOT (565±0.63), total bilirubin (0.305±0.0083), direct bilirubin (0.095±0.0083), SGPT (382.67±1.154) and gamma GT (66.5±0.836) were raised in male rabbit blood parameters. In female rabbit unexpected different results were obtained such as direct bilirubin (8.5±0.836), alkaline phosphatase (5.83±1.036), and gamma GT (8.5±0.836) were found towards the higher side. Whereas, SGOT (18.67±0.96), SGPT (30.83±1.036) and total bilirubin (0.215±0.0083) were towards lower level in female control group.

**Graph 3:** Shows the effect of *A. uva-ursi* extract on cardiac enzymes of rabbits (male & female) in comparison with the control (male & female).

UUF = Female rabbits treated with *A. uva-ursi* extract; UUM = Male rabbits treated with *A. uva-ursi* extract

Urine analysis results were found similar to control and standard drug of pH 8.6 to 9.0 in both sexes. This information is in favor of the use of this drug because it has no drastic effect or change in urine biochemistry parameters.

For performing autopsy of male rabbit organ, 25 mg extract was given to male and female rabbits daily for 3 months. Later autopsy of liver, kidney, heart and stomach were carried out. No pathological changes were observed in male and female rabbits. Our research work supports the previous work done on *A. uva-ursi* by de Arriba *et al.* (2013). In the rabbits injected carbon tetrachloride subcutaneously, no pathological changes were found in heart and stomach tissues. Mild portal inflammation and peri-portal fibrosis with focal macro-vesicular steatosis was observed in liver tissues in *A. uva-ursi* while non-specific pyelonephritis was seen in kidney tissues.

**Graph 4:** Shows the effect of *A. uva-ursi* extract on lipid profile parameters of rabbits (female & male) in comparison with the control (female & male).

UUF = Female rabbits treated with *A. uva-ursi* extract; UUM = Male rabbits treated with *A. uva-ursi* extract

**Graph 5:** Shows the effect of *A. uva-ursi* extract on liver enzymes of rabbits (female & male) in comparison with the control (female & male).

UUF = Female rabbits treated with *A. uva-ursi* extract; UUM = Male rabbits treated with *A. uva-ursi* extract

**CONCLUSION**

Our positive anti-microbial, anti-oxidant, analgesic, anti-inflammatory, diuretic and anti-urolithic results confirms the use of *A. uva-ursi* for treatment of pain, contact dermatitis, urinary tract infection, removal of kidney stones and diuresis. Therefore, our results support the utilization of *A. uva-ursi* in medicine.

**REFERENCES**


