5. RESULTS

5.1 Phyto-chemical and physico-chemical evaluation (Table 5.1a and 5.1b)

Phytochemical evaluation was done for presence of various phytoconstituents. The results of *Hordeum vulgare* were as below.

Table 5.1a: Physico-chemical parameters of *Hordeum vulgare* seeds

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Quality Parameters</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ash value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Total ash value</td>
<td>16.26%</td>
</tr>
<tr>
<td></td>
<td>b. Acid insoluble ash</td>
<td>6.53%</td>
</tr>
<tr>
<td></td>
<td>c. Water soluble ash</td>
<td>2.67%</td>
</tr>
<tr>
<td>2.</td>
<td>Extractive value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Water soluble extractive</td>
<td>13.5%</td>
</tr>
<tr>
<td></td>
<td>b. Alcohol Soluble extractive</td>
<td>5.2%</td>
</tr>
<tr>
<td>3.</td>
<td>Moisture content</td>
<td>4.5%</td>
</tr>
</tbody>
</table>

Table 5.1b: Phyto-chemical screening of *Hordeum vulgare* seeds

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Tests</th>
<th>Positive(+ve)/ Negative(-ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragendorff’s reagent test</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Shinoda test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Fluorescence test</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Haemolytic zone test</td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Fehling test</td>
<td>+ve</td>
</tr>
<tr>
<td>Steroids and triterpenoids</td>
<td>Liberman burchard test</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Salkowski reaction</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Test with gelatin</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Test with lead acetate</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenolics</td>
<td>With FeCl₃</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>With Folin ciocalteu reagent</td>
<td>+ve</td>
</tr>
<tr>
<td>Coumarins</td>
<td>With ammonia</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>With hydroxylamine hydrochloride</td>
<td>-ve</td>
</tr>
<tr>
<td>Quinone glycoside</td>
<td>Borntrager’s test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Modified borntrager’s test</td>
<td>+ve</td>
</tr>
</tbody>
</table>
5.2 Pharmacological studies

5.2.1 Acute toxicity study and Fixation of dosage

The acute toxicity study results indicated that, there was no significance changes in general behavior, any remarkable signs and symptoms of toxicity as well as at all mortality were observed after the administration of a dose up to EHV 5000 mg/kg during observation period. There was no lethality up to a dose of 5.0 g/kg. One-tenth of the maximum dose of extract was selected as an effective dose. Hence, the therapeutic dose was taken as 100 mg/kg (EHV 100), 250 mg/kg (EHV 250) and 500 mg/kg (EHV 500) body weight for the Ethanolic extract of *Hordeum vulgare* seeds.

5.2.2 Anti-urolithiasis and antioxidant activity of *Hordeum vulgare* seeds on Ethylene glycol induced urolithiasis in rats.

5.2.2.1. Preventive study

a) General parameters

In the present study, there was significant decreased in animal weight and urine volume and increase in the dry and wet kidney weight in calculi control animals as compared to normal control animals. These changes were significantly prevented by treatment with standard drug cystone 750 mg/kg (cystone 750), EHV 250 and EHV 500 indicating diuretic activity (Table 5.2). The pH of urine was found acidic in normal control, whereas alkaline in model control animals. Treatment with standard drug Cystone 750 and EHV 500 significantly prevented shift of pH from acidic to alkaline (Table 5.2). EHV 100 and EHV 250 did not produce any significant effect on pH of urine.
Table 5.2: Effect of Ethanolic extract of *Hordeum vulgare* Linn. Seeds on various physical parameters in ethylene glycol (Preventive study) induced renal stone.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL CONTROL</th>
<th>CALCULI INDUCED</th>
<th>EHV 100 TREATED</th>
<th>EHV 250 TREATED</th>
<th>EHV 500 TREATED</th>
<th>CYS 750 TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g/rat)</td>
<td>248.00 ± 6.481</td>
<td>172.16 ± 6.030*</td>
<td>240.00 ± 10.567**</td>
<td>236.66 ± 7.265**</td>
<td>239.16 ± 8.604**</td>
<td>231.66 ± 7.149**</td>
</tr>
<tr>
<td>Urine output (ml/rat/day)</td>
<td>18.19 ± 0.515</td>
<td>17.16 ± 0.535*</td>
<td>19.10 ± 0.500</td>
<td>19.98 ± 0.500</td>
<td>21.69 ± 0.367**</td>
<td>20.89 ± 0.455**</td>
</tr>
<tr>
<td>Ph</td>
<td>6.60 ± 0.107</td>
<td>7.89 ± 0.119*</td>
<td>7.78 ± 0.119</td>
<td>7.14 ± 0.133**</td>
<td>7.32 ± 0.104**</td>
<td>7.07 ± 0.135**</td>
</tr>
<tr>
<td>Wet kidney weight (g)</td>
<td>0.94 ± 0.043</td>
<td>1.21 ± 0.085*</td>
<td>0.85 ± 0.071</td>
<td>0.90 ± 0.045**</td>
<td>0.92 ± 0.049**</td>
<td>0.91 ± 0.056**</td>
</tr>
<tr>
<td>Dry kidney weight (g)</td>
<td>0.18 ± 0.021</td>
<td>0.30 ± 0.028*</td>
<td>0.28 ± 0.018</td>
<td>0.26 ± 0.029**</td>
<td>0.23 ± 0.027**</td>
<td>0.20 ± 0.015**</td>
</tr>
</tbody>
</table>

Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated.
* Significantly different from normal control group, p<0.05
** significantly different from calculi control, p<0.05.
b) Analysis of urine

Chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to wistar rats resulted in hyperoxaluria. The excretion of urolithiasis promoters like calcium, oxalate, inorganic phosphate and uric acid were found significantly high in urine of calculi induced rats. And, Urolithiasis inhibitors like citrate and magnesium concentration were significantly decreased in the urine of calculi induced rats. The renal functioning parameter like urea was observed significantly high in urine of calculi induced animals. The supplementation with standard drug Cystone 750 and EHV 500 significantly decreased the levels of Calcium (Fig. 5.1A), oxalate (Fig. 5.1B), inorganic phosphate (Fig. 5.1C) and uric acid (Fig. 5.2A). There was decreased concentration of urea (Fig. 5.2B) in calculi induced rats treated with ethanolic extract dose dependently. The EHV 250 showed significant reduction in oxalate and uric acid concentration in urine of calculi induced rats. EHV 100 failed to produce significant effect in oxalate and uric acid.

In calculi induced rats, urolithiasis inhibitors like citrate (Fig. 5.3A) and magnesium (Fig. 5.3B) concentration were statistically significantly raised by the standard drug Cystone 750, EHV 250 and EHV 500. The EHV 100 was improving the citrate concentration only. But, not improved the magnesium concentration in urolithiatic rats.
Results

**Calcium in urine**

![Graph A](graph_a.png)

**Oxalate in urine**

![Graph B](graph_b.png)

**Inorg. phosphate in urine**

![Graph C](graph_c.png)

Figure 5.1: Effect of Ethanolic extract of *Hordeum vulgare* seeds (preventive study) on Urine calcium (A), oxalate (B) and Inorg. Phosphate (C) in EG induced Urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. *Significantly different from normal control group, p<0.05 **significantly different from calculi control, p<0.05.
Figure 5.2: Effect of Ethanol extract of *Hordeum vulgare* seeds (preventive study) on Urine uric acid (A) and urea (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.3: Effect of Ethanol extract of *Hordeum vulgare* seeds (Preventive study) on Urine citrate (A) and magnesium (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
c) Analysis of serum

The major constituents of kidney stone like calcium, inorganic phosphate and uric acid concentration were remarkably increased in serum of animals by stone inducing treatment whereas the concentration of magnesium was decreased in calculi induced rats that indicate marked renal damage. Treatment with EHV 500 cause significant reduction in concentration of calcium (Fig. 5.4A), inorganic phosphate (Fig. 5.4B) and uric acid (Fig. 5.5A) in calculi induced animals. Treatment with EHV 250 and EHV 500 reduced the elevated inorganic phosphate level. Whereas EHV 500 (Fig. 5.5B) increased the magnesium levels in calculi induced animals as compared to urolithiatic animals. The renal stone induction caused impairment of kidney functions of the urolithiatic control rats as evident from the markers of glomerular and tubular damage: elevated serum urea, blood urea nitrogen and creatinine level which were significantly increased in urolithiatic rats. The treatment with EHV 500 cause significantly decreased level of kidney function parameters like serum urea (Fig. 5.6A), blood urea nitrogen (Fig. 5.6B) and creatinine (Fig. 5.6C). These effects were comparable to the effect of standard drug cystone 750 on calculi induced rats. The EHV 250 and EHV 100 were fail to produced any significant effect on urea, blood urea nitrogen and creatinine concentration.
Figure 5.4: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on Serum calcium (A) and Inorg. Phosphate (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.5: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on Serum uric acid (A) and magnesium (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

**Urea in serum**

![Graph A](image1.png)

**Blood Urea Nitrogen**

![Graph B](image2.png)

**Creatinine In Serum**

![Graph C](image3.png)

Figure 5.6: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on Serum urea (A), blood urea nitrogen (B) and creatinine (C) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
d) Analysis of kidney homogenate

The calcium (Fig. 5.7A), phosphate (Fig. 5.7B), oxalate (Fig. 5.8A) and uric acid (Fig. 5.8B) level were significantly elevated in kidney homogenate of calculi induced rats. The EHV 500 treatment significantly reduced the levels of all parameters mentioned above in comparison to normal control rats. This effect was comparable to the effect of standard drug cystone 750 on calculi induced rats. The EHV 250 did not produce any significant change in calcium, uric acid and oxalate level in kidney homogenate except phosphate level in calculi induced rats.

![Graph A](image)

![Graph B](image)

Figure 5.7: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on Kidney calcium (A) and Inorg. Phosphate (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.8: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on Kidney oxalate (A) and uric acid (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
e) Analysis of oxidant-antioxidant parameters

For *in vivo* antioxidant activity, ethylene glycol treatment significantly increased malondialdehyde and decreased superoxide dismutase and catalase levels in calculi-induced rats as compared to normal control rats. The treatment with standard Cystone and 500 produced significant reduction in malondialdehyde level (Fig. 5.9B) in calculi induced rats. The EHV 250 and EHV 500 were significantly improved the level of antioxidant enzymes like superoxide dismutase (Fig. 5.10A) and catalase (Fig. 5.10B) as compared to calculi control rats. The EHV 100 also exerted significant reduction of catalase concentration. But EHV 100 did not produce any significant effect on other antioxidant parameters.

![Protein in kidney](A)

![Malondialdehyde in kidney](B)

Figure 5.9: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on antioxidant parameters like Tissue protein (A) and malondialdehyde (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05  ** significantly different from calculi control, p<0.05.
Figure 5.10: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Preventive study) on antioxidant parameters like superoxide dismutase (A) and catalase (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
f) Microscopic observation of Crystalurea

The data of Table 5.3 indicate that, there was significant increase in number of crystals in the urine of model control rats as compare to normal control rats. But 28 days treatment with standard drug cystone 750, EHV 250 and EHV 500 has significantly prevented Crystalurea.

Table 5.3: Effect of test and standard drug (Preventive study) in Crystalurea of EG induced urolithiasis.

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Calculi Control</th>
<th>EHV 100 Treated</th>
<th>EHV 250 Treated</th>
<th>EHV 500 Treated</th>
<th>Cystone treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Histopathology of kidney

Histopathology of kidney section was carried out to study the changes in the cell structure due to ethylene glycol. The examination of kidney section of control animals showed no calcium oxalate deposits or other abnormalities in different segments of nephron (Fig. 5.11a). But in Ethylene glycol treated group, calcium oxalate deposits were abundantly found in different segments of nephron including proximal tubules, loop of Henle, distal tubules; collecting ducts etc. The other abnormalities like renal tubular dilation with epithelial damage were also observed on pathological examination (Fig. 5.11b). However, the kidney of animal treated with EHV 250, 500 and standard cystone showed significant prevention in deposition of calcium oxalate crystals in different segment of kidney and fewer abnormalities observed in kidney cell structure compared to calculi control group of animals (Fig. 5.11d, e and f).
Results

Fig. 5.11 a: Normal Control

Fig. 5.11 b: Calculi Control

Fig. 5.11 c: EHV 100 treated

Fig. 5.11 d: EHV 250 treated

Fig. 5.11 e: EHV 500 treated

Fig. 5.11 f: Standard Cystone treated
5.2.2.2 Reversal study

a) General parameters

Table 5.4 represents the general parameters. Ethylene glycol intake significantly decreased body weight and urine volume in calculi control animals as compared to normal control animals. Treatment with standard Cystone 750 and EHV 500 after 2 weeks of ethylene glycol ingestion significantly increased body weight and urine volume as compared to calculi control animals. Urinary pH was found acidic in normal control animals and alkaline in calculi control animals. This increase in pH was also significantly reversed by treatment with standard cystone, EHV 250 and EHV 500. The EHV 100 did not show any significant effect on pH. Similarly there was significant increase in the dry and wet kidney weight in calculi control group as compared to normal control group. These changes were significantly reversed by 14 days treatment with standard Cystone and EHV 500.
Table 5.4: Effect of Ethanolic extract of *Hordeum vulgare* Linn. Seeds on various physical parameters in ethylene glycol (Reversal study) induced renal stone.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL CONTROL</th>
<th>CALCULI INDUCED</th>
<th>EHV 100 TREATED</th>
<th>EHV 250 TREATED</th>
<th>EHV 500 TREATED</th>
<th>CYS 750 TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g/rat)</td>
<td>248.00 ± 6.481</td>
<td>172.16 ± 6.030*</td>
<td>236.83 ± 10.167**</td>
<td>232.50 ± 7.270**</td>
<td>245.50 ± 9.965**</td>
<td>231.66 ± 7.149**</td>
</tr>
<tr>
<td>Urine output (ml/rat/day)</td>
<td>18.19 ± 0.515</td>
<td>17.16 ± 0.535*</td>
<td>19.78 ± 0.365</td>
<td>21.32 ± 0.480</td>
<td>22.60 ± 0.440**</td>
<td>20.89 ± 0.455**</td>
</tr>
<tr>
<td>Ph</td>
<td>6.60 ± 0.107</td>
<td>7.89 ± 0.119*</td>
<td>7.98 ± 0.019</td>
<td>7.35 ± 0.176</td>
<td>7.18 ± 0.144**</td>
<td>7.07 ± 0.135**</td>
</tr>
<tr>
<td>Wet kidney weight (g)</td>
<td>0.94 ± 0.043</td>
<td>1.21 ± 0.085*</td>
<td>0.81 ± 0.064</td>
<td>0.87 ± 0.043</td>
<td>0.99 ± 0.054**</td>
<td>0.91 ± 0.056**</td>
</tr>
<tr>
<td>Dry kidney weight (g)</td>
<td>0.18 ± 0.021</td>
<td>0.30 ± 0.028*</td>
<td>0.28 ± 0.018</td>
<td>0.21 ± 0.023</td>
<td>0.21 ± 0.027**</td>
<td>0.20 ± 0.015**</td>
</tr>
</tbody>
</table>

Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated.

* Significantly different from normal control group, p<0.05
** significantly different from calculi control, p<0.05.
b) Analysis of urine

In the reversal study, there was an increased exertion of kidney stone promoters like calcium, oxalate and inorganic phosphate significantly higher in animals due to administration of ethylene glycol (0.75% v/v). The treatment with EHV 500 was significant reverse this rise in promoters level as compared to calculi control animals (Fig. 5.12 A, B and C). The kidney stone promoter like uric acid and urea were found significantly high in calculi induced animals. While, with the treatment of extract EHV 500, significantly reverse the concentration of uric acid (Fig. 5.13A) and urea (Fig. 5.1B) respectively in comparison with calculi control animals. EHV 250 was also showed significantly reduced level of urea in calculi induced animals. EHV 100 did not produce any significant effect on these parameters. Such effects were comparable to the effect of standard drug cystone in calculi control rats.

The calculi control rats showed significantly decreased in citrate and magnesium level as compared to normal control rats. There was Significant reversal effect was observed with treatment of standard cystone and EHV 500 on urolithiasis inhibitors like citrate (Fig. 5.14A) and magnesium (Fig. 5.14B). Here, EHV 100 and 250 did not produce significant change in such parameters of calculi induced rats. The renal functioning parameter like urea was found significantly high in the urine of urolithiatic rats as compare to normal control rats. The EHV 250 and 500 produced significantly reversed the level of urea in urolithiatic animal. Such effect was also found with the standard drug cystone on calculi induced rats.
Figure 5.12: Effect of Ethanolistic extract of *Hordeum vulgare* seeds (Reversal study) on urine calcium (A), oxalate (B) and Inorg. Phosphate (C) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.13: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Reversal study) on Urine uric acid (A) and urea (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.14: Effect of Ethanol extract of *Hordeum vulgare* seeds (Reversal study) on Urine citrate (A) and magnesium (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculus control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
c) Analysis of serum

The calculi induced animals showed significant high concentration of calcium, inorganic phosphate and uric acid (Fig. 5.15A, B and 5.16A) in comparison to normal control animals. The magnesium concentration was significantly decreased in calculi induced animals (Fig. 5.16B). The standard cystone and EHV 500 reversed the effect on such mentioned parameters in serum. Here EHV 100 and EHV 250 did not produce any significant effects on such mentioned parameters. The kidney functioning parameters like urea (Fig. 5.17A); blood urea nitrogen (Fig. 5.17B) and creatinine (Fig. 5.17C) level were observed higher in calculi induced animals. The treatment with standard cystone, EHV 500 and 250 produced a significant reduction in urea, blood urea nitrogen and creatinine level when compared with calculi control animals.
Figure 5.15: Effect of Ethanol extract of *Hordeum vulgare* seeds (Reversal study) on serum calcium (A), and Inorg. Phosphate (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

Figure 5.16: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Reversal study) on Serum uric acid (A) and magnesium (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.17: Effect of Ethanolic extract of *Hordeum vulgare* seeds (reversal study) on Serum urea (A), blood urea nitrogen (B) and creatinine (C) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
d) Analysis of kidney homogenate

The calculi induced animals exhibited significantly higher calcium, phosphate, uric acid and oxalate level in kidney homogenate. The levels of above mentioned parameters like calcium (Fig. 5.18A), phosphate (Fig. 5.18B), uric acid (Fig. 5.19A) and oxalate (Fig. 5.19B) were significantly reversed by the treatment with the standard cystone and EHV 500 as compare to calculi control animals. The EHV 100 and EHV 250 were failed to exhibit significant reduction in calcium, uric acid and oxalate level in kidney homogenate.

![Graph A: Calcium in kidney](image)

![Graph B: Inorg. phosphate in kidney](image)

**Figure 5.18:** Effect of Ethanolic extract of *Hordeum vulgare* seeds (Reversal study) on Kidney calcium (A) and Inorg. Phosphate (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

**Uric acid in kidney**

![Graph A](image)

**Oxalate in kidney**

![Graph B](image)

**Figure 5.19**: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Reversal study) on Kidney uric acid (A) and oxalate (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
e) **Analysis of oxidant-antioxidant parameters**

For *in vivo* antioxidant activity, ethylene glycol treatment significantly increased malondialdehyde and decreased superoxide dismutase and catalase levels in calculi-induced rats as compared to normal control rats. The treatment with standard Cystone 750, EHV 250 and 500 produced significant reduction in malondialdehyde (Fig. 5.20B) and increased level of superoxide dismutase (Fig. 5.21A) and catalase (Fig. 5.21B) as compared to calculi control rats.

![Graph A: Protein in kidney](image)

![Graph B: Malondialdehyde in kidney](image)

**Figure 5.20:** Effect of Ethanolic extract of *Hordeum vulgare* seeds (Reversal study) on antioxidant parameters like Tissue protein (A) and malondialdehyde (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. *Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

Figure 5.21: Effect of Ethanolic extract of *Hordeum vulgare* seeds (Reversal study) on antioxidant parameters like superoxide dismutase (A) and catalase (B) in EG induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantlly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
f) Microscopic observation of Crystalurea

Table 5.5 indicates the presence of crystals in urine of animals. After four week treatment intake of ethylene glycol, Crystalurea was significantly increased in model control as compared to normal control. But it was significantly reversed by 14 days treatment with standard cystone 750, EHV 250 and 500.

Table 5.5: Effect of test and standard drug (Reversal study) in Crystalurea of EG induced urolithiasis.

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Calculi Control</th>
<th>EHV 100 Treated</th>
<th>EHV 250 Treated</th>
<th>EHV 500 Treated</th>
<th>Cystone treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>++++</td>
<td>++++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Histopathology of kidney

Histopathology of kidney section was carried out to study the changes in the cell structure due to ethylene glycol. The examination of kidney section in control animals showed no calcium oxalate deposits or other abnormalities in different segments of nephron (Fig. 22a). But in Ethylene glycol treated group, calcium oxalate deposits were abundantly found in different segments of nephron, including proximal tubules, loop of Henle, distal tubules; collecting ducts etc. the other abnormalities like renal tubular dilation with epithelial damage were also observed on pathological examination (Fig. 22b). However, the kidney of animal treated with standard cystone, EHV 250 and 500 showed significant prevention in deposition of calcium oxalate crystals in different segment of kidney and fewer abnormalities observed in kidney cell structure compared to calculi control group of animals (Fig. 22d,e and f).
Results

Fig. 5.22 a: Normal Control

Fig. 5.22 b: Calculi Control

Fig. 5.22 c: EHV 100 treated

Fig. 5.22 d: EHV 250 treated

Fig. 5.22 e: EHV 500 treated

Fig. 5.22 f: Standard Cystone treated
5.2.3 Effect of Ethanolic extract of *Hordeum vulgare* Linn. Seeds on glycolic acid induced urolithiasis in rats.

*a) General parameters*

The urolithiasis induced by the supplementation of glycolic acid with normal commercial diet for the 42 days. Here, various physical parameters like body weight, volume of urine, pH of urine and wet kidney weight of rats were measured at the end of the treatment (Table 5.6). The body weight and urine volume were significantly decreased in calculi induced rats as compared to normal control rats. Treatment with standard drug cystone and EHV 500 showed significantly improve the body weight; while treatment with EHV 500 was significantly increase the urine volume. The standard drug Cystone 750 and EHV 250 did not produce any significant effect on volume of urine in calculi induced animals when compared with control animals. The pH of urine of calculi controlled animals were significantly increased as compared to normal control rats in which, there was slightly acidic pH was observed. Only standard drug cystone having significant effect on pH. While, EHV extract did not produce any significant effect in calculi induced animals. There was a significant increase in the kidney weight of animals receiving 3% glycolic acid which was significantly reduced by the treatment with Cystone and the EHV 500.
Table 5.6: Effect of Ethanolic extract of *Hordeum vulgare* Linn. Seeds on various physical parameters in Glycolic acid induced renal stone.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL CONTROL</th>
<th>CALCULI INDUCED</th>
<th>EHV 100 TREATED</th>
<th>EHV 250 TREATED</th>
<th>EHV 500 TREATED</th>
<th>CYS 750 TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g/rat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>230.33 ± 2.667</td>
<td>180.01 ± 5.323*</td>
<td>195.03 ± 6.831</td>
<td>218.50 ± 6.927</td>
<td>222.00 ± 6.028**</td>
<td>226.66 ± 9.280**</td>
</tr>
<tr>
<td>Urine output (ml/rat/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.66 ± 1.333</td>
<td>11.83 ± 0.872*</td>
<td>12.88 ± 1.195</td>
<td>18.50 ± 0.992</td>
<td>22.66 ± 2.011**</td>
<td>21.50 ± 1.057</td>
</tr>
<tr>
<td>pH</td>
<td>6.41 ± 0.580</td>
<td>7.55 ± 0.600*</td>
<td>7.01 ± 0.130</td>
<td>6.88 ± 0.530</td>
<td>6.70 ± 0.520</td>
<td>6.65 ± 0.950**</td>
</tr>
<tr>
<td>Wet kidney weight (g)</td>
<td>1.01 ± 0.360</td>
<td>1.21 ± 0.310</td>
<td>0.81 ± 0.220</td>
<td>0.85 ± 0.062</td>
<td>0.89 ± 0.143**</td>
<td>0.93 ± 0.260**</td>
</tr>
</tbody>
</table>

Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated.
* Significantly different from normal control group, p<0.05
** significantly different from calculi control, p<0.05.
b) Analysis of urine

Due to the glycolic acid induced hyperurolxaluria (urolithiasis), there was grossly increased level of urinary excretion of urolithiatic promoters like calcium, oxalate, inorganic phosphate and uric acid and, the urolithiasis inhibitor like citrate was significantly decreased in calculi induced animals as compared to normal control animals. The supplementation with EHV 500 significantly decreased the urinary excretion of calcium (Fig. 5.23A), oxalate (Fig. 5.23B), inorganic phosphate (Fig. 5.23C) and uric acid (Fig. 5.24A) in calculi induced animals. The citrate level was significantly improved by EHV 500 (Fig. 5.24C) in urolithiatic animals with respect to calculi control animals. This effect was compared to the effect standard drug cystone 750 on calculi induced animals.

The calculi control rats exhibited significantly higher urea level when compared with normal control rats. The chronic treatment with EHV 500 produced significantly decrease in urea level in calculi induced rats (Fig. 5.24B). The standard drug cystone 750 was exhibited the same effect on urinary excretion of urea. Here EHV 100 and 250 did not show any significant effect on such parameters of urine.
Figure 5.23: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Urine calcium (A), Oxalate (B) and Inorg. Phosphate (C) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

Figure 5.24: Effect of Ethanol extract of *Hordeum vulgare* seeds on Urine uric acid (A), urea (B) and citrate (C) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

c) Analysis of serum
The serum calcium and inorganic phosphate concentration were remarkably increased in calculi-induced rats as compared to the normal control rats. Treatment with standard drug and EHV 500 significantly lowered the elevated level of serum calcium (Fig. 5.25A) and inorganic phosphate (Fig. 5.25B) in calculi induced animals.

Renal stone induction caused impairment of renal functions of the calculi control animals as evident from the markers of glomerular and tubular damage as reflected by the significantly elevated levels of serum uric acid, urea and blood urea nitrogen in calculi induced animals. The chronic treatment with EHV 500 significantly decreased the level of uric acid (Fig. 5.26A) in calculi animals. The treatment with EHV 100 and 250 did not produce any significant effect on level of urea (Fig. 5.26B) and blood urea nitrogen (Fig. 5.26C) in comparison with calculi control animals. The standard drug Cystone also produced significant effect on such renal function parameters.
Figure 5.25: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum calcium (A) and Inorg. Phosphate (B) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.26: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum uric acid (A), urea (B) and blood urea nitrogen (C) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
d) Analysis of kidney homogenate

The deposition of the crystalline components in the renal tissue namely calcium, phosphate, uric acid and oxalate level were prominently increased in kidney homogenate of calculi induced animals group. The treatment with cystone 750 and EHV 500 significantly decreased the levels of all parameters mentioned above (Fig. 5.27A & B and Fig. 5.28A & B) when compared with calculi controlled animals. The EHV 100 and 250 failed to exhibit significant effect on calcium, uric acid and oxalate level in kidney homogenate except phosphate level in calculi induced animals as compared with calculi control animals.

![Calcium in kidney](image)

![Inorg. phosphate in kidney](image)

**Figure 5.27: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Kidney calcium (A) and Inorg. Phosphate (B) in GA induced urolithiasis.** Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.28: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Kidney uric acid (A) and oxalate (B) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
e) **Analysis of oxidant-antioxidant parameters**

For *in vivo* antioxidant activity, glycolic acid supplementation produced significant increased malondialdehyde and decreased superoxide dismutase and catalase levels in calculi-induced animals as compared to normal control animals. The treatment with EHV 500 produced significant reduction in malondialdehyde (Fig. 5.29B) and showed significant elevation in the superoxide dismutase (Fig. 5.30A) and catalase levels (Fig. 5.30B) as compared to calculi control animals. The standard cystone also exhibit the same effect as the test on antioxidant enzyme level.

![Protein in kidney](A)

![Malondialdehyde in kidney](B)

**Figure 5.29:** Effect of Ethanolic extract of *Hordeum vulgare* seeds on antioxidant parameters like tissue protein (A) and malondialdehyde (B) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.30: Effect of Ethanolic extract of *Hordeum vulgare* seeds on antioxidant parameters like superoxide dismutase (A) and catalase (B) in GA induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Calculi control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
5.2.4 Surgical method of urolithiasis

Table 5.7 represents the general variables obtained after the introduction of CaOx crystals. After two weeks of insertion of stone in the bladder, there was significant decreased in body weight of calculi control animals. The weight loss was not observed in sham operated animals and was significantly prevented by the treatment with standard drug cystone and the EHV 250. Average daily water intake was significantly reduced in the calculi control animals as compare to normal control and sham operated animals, whereas improvement in water intake was observed with the treatment of standard and test drugs. There was also significant decreased in urine output in calculi control animals but treatment with test as well as standard drugs significantly caused diuresis. Slight shift of urine pH from acidic to alkaline was observed with calculi control animals as compared to normal control animals. The treatment with standard cystone and EHV significantly prevented this change of pH.

The X-ray examination of urinary bladder at the beginning and at the end of experimental period was carried out. The x-ray examination of urinary bladder at the end of treatment, there was clearly observed increased in growth of crystals in calculi control animals. Animals treated with the extract and the standard drug showed inhibition of the crystal growth in bladder. This crystal growth was significantly prevented by the treatment of EHV 500 and standard Cystone (Fig. 5.31a-f).
Results

Fig. 5.31 a: Sham Operated (Before)

Fig. 5.31 b: Sham Operated (After)

Fig. 5.31 c: Calculi Controlled (before)

Fig. 5.31 d: Calculi Controlled (After)

Fig. 5.31 e: EHV 250 mg/kg treated (Before)

Fig. 5.31 f: EHV 250 mg/kg treated (After)
Results

Fig. 5.31 g: EHV 500 mg/kg treated (Before)

Fig. 5.31 h: EHV 500 mg/kg treated (After)

Fig. 5.31 i: Cystone treated (Before)

Fig. 5.31 j: Cystone treated (After)
Table 5.7: Effect of Ethanolic extract of *Hordeum vulgare* Linn. Seeds on various physical parameters in surgically induced renal stone.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>NORMAL CONTROL</th>
<th>SHAM-OPERATED</th>
<th>CALCULI CONTROL</th>
<th>EHV 250 TREATED</th>
<th>EHV 500 TREATED</th>
<th>CYS 750 TREATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g/rat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>325.00 ± 13.660</td>
<td>295.00 ± 6.960</td>
<td>257.01 ± 12.187*</td>
<td>196.05 ± 9.320</td>
<td>287.14 ± 7.201**</td>
<td>297.66 ± 11.640**</td>
</tr>
<tr>
<td>Water intake (ml/day/rat)</td>
<td>26.66 ±1.498</td>
<td>23.16 ± 1.167</td>
<td>16.16 ± 0.792*</td>
<td>24.66 ± 0.715</td>
<td>26.33 ± 0.667</td>
<td>25.50 ± 0.563**</td>
</tr>
<tr>
<td>Urine output (ml/rat/day)</td>
<td>20.96 ± 0.730</td>
<td>20.22 ± 0.610</td>
<td>13.29 ± 0.635*</td>
<td>19.11 ± 0.735**</td>
<td>12.99 ± 0.395**</td>
<td>18.37 ± 0.530**</td>
</tr>
<tr>
<td>pH</td>
<td>6.51 ± 0.168</td>
<td>6.47 ± 0.157</td>
<td>7.73 ± 0.121*</td>
<td>6.99 ± 0.221**</td>
<td>7.42 ± 0.177**</td>
<td>6.86 ± 0.107**</td>
</tr>
</tbody>
</table>

Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Sham operated; GP III = Calculi control; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated.

* Significantly different from normal control group, p<0.05
** Significantly different from calculi control, p<0.05.
b) Analysis of urine

At the end of study period, the urine of surgically induced urolithiatic control animals showed significantly higher elimination of calcium, oxalate, phosphate, uric acid as compare to normal control animals. There were significantly decreased elimination of citrate and magnesium of calculi control animals as compared to normal control animals. Sham operated animals showed the similar results to normal control animals. The treatment with standard drug cystone 750 and EHV 500 significantly decreased the elimination of calcium (Fig. 5.32A), oxalate (Fig. 5.32B), phosphate (Fig. 5.32C) and uric acid (Fig. 5.33A) when compared with calculi control animals. Whereas, the treatment with standard drug cystone, produced significantly increased the levels of citrate (Fig. 5.33B) and magnesium (Fig. 5.33C). The treatment with EHV 100, 250 and 500 were failed to produce significant effect on such urolithiasis inhibitory parameters.
Results

Figure 5.32: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Urine calcium (A), oxalate (B) and Inorg. Phosphate (C) in surgically induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Sham operated; GP III = Calculi control; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.33: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Urine uric acid (A), citrate (B) and magnesium (C) in surgically induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Sham operated; GP III = Calculi control; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
c) Analysis of serum
The kidney stone inducers like calcium, phosphate and uric acid concentration significantly increased in serum of calculi induced rats as compared to normal control rats. Urolithiatic inhibitors like Magnesium concentration decreased significantly in calculi induced rats. The sham operated group of animals showed the parallel effect as compared to normal control rats. On completion of drug therapy, the concentration of kidney stone inducers were significantly reversed by the standard drug Cystone 750 and EHV 500 as compared to calculi induced rats (Fig. 5.34B & C). The calcium level did not significantly reverse by the EHV at any dose (Fig. 5.34A). The concentration of magnesium was not significantly increased by the EHV (Fig. 5.35A).

The renal functioning parameters like urea and blood urea nitrogen level were significantly elevated in calculi induced rats. The treatment with standard drug cystone produced significantly reduction in elevated levels of urea and blood urea nitrogen as compared to calculi control animals. Here, the EHV did not produced any significant effects on urea and blood urea nitrogen levels in calculi induced rats as compared to calculi control rats (Fig. 5.35B & C).
Results

Figure 5.34: Effect of Ethanol extract of *Hordeum vulgare* seeds on Serum calcium (A), Inorg. Phosphate (B) and uric acid (C) in surgically induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Sham operated; GP III = Calculi control; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.35: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum magnesium (A), urea (B) and blood urea nitrogen (C) in surgically induced urolithiasis. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Sham operated; GP III = Calculi control; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Cystone (750 mg/kg) treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
5.2.5 Gentamicin induced nephrotoxicity

a) General parameters

The current study is about the nephrotoxicity induced by Gentamicin. The data showed that, the changes in body weight was decreased in Gentamicin treated animals compare to normal control animals. The animals treated with EHV 500 produced significant improvement of body weight (Fig. 5.36A). While, EHV 100 and 250 did not showed significant change in body weight of Gentamicin treated animals. In Gentamicin treated animals, kidney weight was considerably increased compared to normal control animals. The treatment with EHV 250 and 500 showed significant decrease in kidney weight (Fig. 5.36B). Here EHV 100 did not show any significant effect on kidney weight of nephrotoxic animals.

Figure 5.36: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Body weight (A) and Kidney weight (B) of Gentamicin induced nephrotoxicity. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Gentamicin control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
b) Analysis of serum

In Gentamicin treated group of animals the concentration of renal function parameters like serum urea, blood urea nitrogen and creatinine were considerably increased as compared to normal control animals, this indicates sever nephrotoxicity. Animals treated with EHV 250 and 500 showed significant decrease in concentration of serum urea (Fig. 5.37A) and blood urea nitrogen (Fig. 5.37B) compared to Gentamicin treated nephrotoxic animals. Here, EHV 100 was failed to produce significant effect on serum urea and blood urea nitrogen. The serum creatinine level was significantly decreased in animals treated with the EHV 100, 250 and 500 (Fig. 5.37C).
Figure 5.37: Effect of Ethanol extract of *Hordeum vulgare* seeds on Serum urea (A), blood urea nitrogen (B) and creatinine (C) of Gentamicin induced nephrotoxicity. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Gentamicin control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
c) Analysis of oxidant-antioxidant parameters

Here, antioxidant parameters were measured in kidney homogenate of all animals. There were significant decrease in the levels of protein, superoxide dismutase, reduced glutathione and catalase in Gentamicin treated nephrotoxic animals in comparison with normal control animals. The treatment with EHV 100, 250 and 500 showed significantly decrease in the level of protein (Fig. 5.38A). The EHV 100 and 250 were significantly increased the level of superoxide dismutase in nephrotoxic animals (Fig. 5.38C). Here, EHV 500 was not increased the superoxide dismutase level significantly on Gentamicin treated nephrotoxic animals as compared to normal control animals. The level of reduced glutathione was also significantly increased by the treatment with EHV 250 and 500 (Fig. 5.39A). And the concentration of catalase was significantly increased by the EHV 500 (Fig. 5.39B). Here EHV 100 and 250 did not produced significant effect on concentration of catalase. The concentration of malondialdehyde was significantly increased in Gentamicin treated animals in comparison with normal control animals. The EHV 100, 250 and 500 were significantly decreased the concentration of malondialdehyde in Gentamicin treated animals (Fig. 5.38B). The presented data showed that the extract having the potent anti-oxidant activity in Gentamicin induced nephrotoxicity.
Figure 5.38: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Tissue protein (A), malondialdehyde (B) and superoxide Dismutase (C) of Gentamicin induced nephrotoxicity. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Gentamicin control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.39: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Kidney reduced glutathione (A) and catalase (B) of Gentamicin induced nephrotoxicity. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Gentamicin control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Histopathological examination

The microscopic examination of kidney from normal control rats showed no structural alteration in renal tissue. The glomerular and tubular changes appear unremarkable observed in normal control rats. The tubular structures were largely intact without the presence of any mononuclear infiltrates in the interstitium (Fig. 5.40a). In GM group, there were seen diffuse glomerular congestion, degeneration of tubular epithelial cell and peritubular congestion. The blood vessels congestion and scattered mononuclear inflammatory cell observed within the interstitium. The glomerular changes were quite marked (Fig. 5.40b). Concurrent treatment with the EHV was found to reduce such changes in kidney histology induced by Gentamicin.

In EHV 100 treated animals, the renal tubular cells showed varying degrees of dilatation with hyaline cast formation in the lumen and the degeneration of tubular epithelial cells, peritubular congestion and cell necrosis (Fig. 5.40c). Treatment with EHV 250 and 500 showed renal parenchyma with intact architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels were dilated and glomerular congestion within the interstitium. There were also seen few scattered mononuclear inflammatory infiltration seen within the interstitium (Fig. 5.40d & e). According to the pathological result it can be inferred that extracts of Hordeum vulgare seeds had protective effect against degenerative injury caused by Gentamicin.
Fig. 5.40 a: Normal Control

Fig 4.40 b: Gentamicin treated

Fig. 5.40 c: GM + EHV 100 treated

Fig. 5.40 d: GM + EHV 250 treated

Fig 4.40 e: GM + EHV 500 treated
5.2.6 Streptozotocin induced Type I diabetic nephropathy

*a) General parameters*

In this study, the diabetic nephropathy induced by the intraperitoneal injection with Streptozotocin (STZ). All the rats produced well-developed sign and symptoms of uncontrolled type I diabetes mellitus after STZ administration, that is, hyperglycemia, glycosuria, polyurea, increased water consumption, and weight loss. The rats received STZ, showed significant reduced body weight, increased food and water intake when compared with the normal control rats. The treatment with the EHV 500 produced significantly increased body weight (Fig. 5.41A). The treatment with EHV 250 and 500 were significantly decreased food and water intake (Fig. 5.41B & C) as compared to diabetic control rats. The effect was comparable to standard drug insulin (5 U/kg) on STZ induced diabetic rats.
Results

Figure 5.41: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Body weight (A), food intake (B) and water intake (C) in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
b) Analysis of serum

STZ-induced diabetic nephropathy rats exhibited significantly higher serum glucose level and decreased the level of insulin in diabetic rats as compare with non-diabetic rats during the experimental period. The treatment with standard drug insulin 6 U/kg and the test drug EHV showed significantly increase serum glucose level in dose dependent manner (Fig. 5.42A) as compared with diabetic controlled rats. The serum insulin level was significantly elevated by the treatment with the EHV 500 (Fig. 5.42B) as compared with diabetic controlled rats. This effect was comparable to the effect of standard insulin on STZ-induced diabetic nephropathy. The EHV 100 and 250 did not produce any significant effect on serum insulin level of diabetic rats.

![Glucose in serum](A)

![Insulin in serum](B)

Figure 5.42: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum glucose (A) and insulin (B) in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
b). Analysis of renal function parameters

During the observation period, STZ-induced diabetic rats showed a marked increase in 24 hr urinary protein excretion as compared with normal control rats. The treatment with EHV 250 and 500 significantly decreased the excretion of urine protein in diabetic rats (Fig. 5.43A). The standard drug insulin was also showed such significant reduction of 24 hr urinary protein excretion. There were significant increase serum urea and creatinine levels in STZ-induced diabetic rats when compared with the normal control rats. The treatment with standard drug insulin and EHV 500 significantly decreased levels of serum urea (Fig. 5.43B) and creatinine (Fig. 5.43C) as compared to diabetic control rats. The EHV 100 and 250 did not produce significant change in serum urea and creatinine level.
Results

**Protein in urine**

![Graph showing protein concentration in urine for different groups.](A)

**Urea in serum**

![Graph showing urea concentration in serum for different groups.](B)

**Creatinine in serum**

![Graph showing creatinine concentration in serum for different groups.](C)

Figure 5.43: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Urine protein (A), Serum urea (B) and creatinine (C) in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
c) Analysis of lipid parameters

The STZ-induced diabetic nephropathic rats produced significant increase in serum triglyceride, Cholesterol, LDL-Cholesterol, VLDL-Cholesterol and a significant decrease in HDL-Cholesterol which resulted increase atherogenic index at significant extend as compared to non diabetic control rats. The treatment of rats with EHV 250 and 500 produced significant decreased in serum triglyceride (Fig. 5.44A), Cholesterol (Fig. 5.44B), LDL-Cholesterol (Fig. 5.44C), VLDL-Cholesterol (Fig. 5.45A) and atherogenic index (Fig. 5.45B). While, significant increased in HDL-cholesterol (Fig. 5.45C) level of diabetic nephropathic rats when compared to non diabetic control rats. Such effects were comparable to the effect of standard dug insulin on diabetic nephropathic rats.
Results

Figure 5.44: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum triglyceride (A), cholesterol (B) and LDL-cholesterol (C) in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
Results

Figure 5.45: Effect of Ethanol extract of *Hordeum vulgare* seeds on Serum VLDL-cholesterol (A), HDL-cholesterol (B) and atherogenic index (C) in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
c) Analysis of oxidant-antioxidant parameters

STZ-induced diabetic nephropathic rats showed significant increased malondialdehyde and decreased superoxide dismutase, reduced glutathione and catalase levels in diabetic control animals as compared to normal animals. The treatment with EHV 500 produced significant reduction in malondialdehyde (Fig. 5.46A) and significantly increased level of superoxide dismutase (Fig. 5.46B), reduced glutathione (Fig. 5.47A) and catalase (Fig. 5.47B) as compared to diabetic control group. The standard drug insulin was also showed the same effect on the diabetic animals.

![Malondialdehyde in kidney](image)

![Superoxide dismutase in kidney](image)

Figure 5.46: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Kidney malondialdehyde (A) and superoxide dismutase (B in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
Results

Figure 5.47: Effect of Ethanol extract of *Hordeum vulgare* seeds on kidney reduced glutathione (A) and catalase (B) in STZ induced type I diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Protamine zinc insulin treated (6 unit/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
Histopathological studies of kidney

The present study indicates that STZ-induced diabetes is sufficient to induced early histological changes in the kidney of animal. The section from normal control showed normal histology of kidney of rat with no pathological changes. The proximal convoluted tubules, distal convoluted tubules and renal corpuscles with glomerular capsules are very clear and prominent. The outer parietal layer of Bowman’s capsule appeared normal with intact epithelium (Fig. 5.48a).

Histopathological changes in kidney of diabetic rat showed significant changes in normal architecture of kidney. The signs of tubular necrosis with loss of their brush border of glomerular capsules. The epithelial lining of Bowman’s capsules was observed highly disrupted (Fig. 5.48b). These changes were found to be significantly reduced in kidneys of the experimental group treated with EHV 500 (Fig. 5.48d) and standard drug insulin (Fig. 5.48e). The Standard drug and EHV 500 treatment showed considerable improvement in glomeruli and tubules. Bowman’s capsule showed comparatively less disturbed and observed intact architecture. The group of rats received low dose of extract 250 showed moderate improvement in the tubular and glomerular morphology (Fig. 5.48c).
Fig. 5.48 a: Normal Control

Fig. 5.48 b: Diabetic Control

Fig. 5.48 c: EHV 250 treated

Fig. 5.48 d: EHV 500 treated

Fig. 5.48 e: Standard Insulin treated
5.2.7 Streptozotocin-Nicotinamide induced Type II diabetic nephropathy

a) General parameters

In present study, type II diabetic nephropathy was induced by the administration of Streptozotocin and nicotinamide consecutively. In the present study, rats which received STZ-nicotinamide exhibited a significant decrease in body weight. Due to the diabetes, there was significantly increased food and water intake as compared to normal control rats. Type 2 diabetic rats when treated with Standard Glibenclamide 5 mg/kg and EHV 500 significantly increased body weight (Fig. 5.49A) and water intake (Fig. 5.49B) when compared with diabetic control rats. The EHV did not exhibit the significant effect on food intake in diabetic rats (Fig. 5.49C). The EHV 100 was failed to produce significant effect on these mentioned parameters.
Figure 5.49: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Body weight (A), food intake (B) and water intake (C) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
b) Analysis of serum

The experimental rats received STZ-nicotinamide showed a significant hyperglycemia associated with hypoinsulinemia in rats. Treatment with EHV significantly decreased the level of serum glucose (Fig. 5.50A) in dose dependent manner. The EHV did not produce any significant effect on serum insulin level (Fig. 5.50B) as compared with diabetic control groups. The treatment with the standard drug Glibenclamide 5 mg/kg were produced significant effect on the both serum glucose and insulin levels in diabetic animals.

![Glucose in serum](image)

**Figure 5.50:** Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum glucose (A) and insulin (B) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
**c) Analysis of renal function parameters**

The renal functioning parameters like serum urea, creatinine and urine protein were measured in diabetic rats. In STZ-nicotinamide induced type 2 diabetic nephropathy the animals showed significant increased level of serum urea, creatinine and 24 hr urine protein as compared with non diabetic control rats. Treatment with EHV 500 significantly decreased level of serum urea (Fig. 5.51A), creatinine (Fig. 5.51B) and 24 hr urine protein (Fig. 5.51C) levels when compared with diabetic control rats. EHV 100 and 250 were failed to produce any significant effect on such renal function parameters. The standard drug Glibenclamide produced significant effect on serum urea, creatinine and 24 hr urine protein concentration in comparison to diabetic rats.
Figure 5.51: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum urea (A), creatinine (B) and urine protein (C) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
d) Analysis of Lipid parameters
STZ-nicotinamide induced diabetic rats produced a significant rise in serum triglyceride, Cholesterol, LDL-Cholesterol, VLDL-cholesterol and thus Atherogenic index while decrease in HDL level when compared with non diabetic control rats. Treatment with EHV 500 exhibited significant decrease in triglyceride (Fig. 5.52A), Cholesterol (Fig. 5.52B), LDL-cholesterol (Fig. 5.52C), VLDL-cholesterol (Fig. 5.53A) and atherogenic index (Fig. 5.53C) in diabetic nephropathic rats when compared with diabetic control rats. Treatment with EHV 250 and 500 caused a significant declined in HDL-cholesterol (Fig. 5.53B). The HDL-cholesterol level was significantly increased by the EHV 500 in diabetic nephropathic rats when compared with diabetic control rats. The effect was comparable to the effect of standard drug Glibenclamide on STZ-nicotinamide induced type II diabetic nephropathic rats. Here, EHV 100 was failed to produce significant effect on such parameters.
Figure 5.52: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum triglyceride (A), cholesterol (B) and LDL-cholesterol (C) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Results

Figure 5.53: Effect of Ethanolic extract of Hordeum vulgare seeds on Serum VLDL-cholesterol (A), HDL-cholesterol (B) and atherogenic index (C) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
c) **Analysis of oxidant-antioxidant parameters**

STZ-nicotinamide induced type 2 diabetic nephropathic rats exhibited significantly higher concentration of malondialdehyde in kidney tissue when compared to normal control rats. Treatment with EHV 250 and 500 were significantly decreased malondialdehyde concentration when compared with diabetic control animals (Fig. 5.54A). This effect was comparable to the effect of Glibenclamide on STZ-nicotinamide induced diabetic group.

STZ-nicotinamide induced type 2 diabetic rats showed significant reduction in superoxide dismutase, reduced glutathione content and catalase in kidney tissue when compared with normal control rats. Treatment with EHV 500 showed significantly rise in the levels of superoxide dismutase (Fig. 5.54B), reduced glutathione (Fig. 5.55A) and catalase (Fig. 5.55B) level when compared with diabetic control rats. However, treatment with EHV did not significantly change the superoxide dismutase levels when compared with diabetic control rats.
Results

Malondialdehyde in kidney

![Graph showing Malondialdehyde levels in kidney across different groups.](A)

Superoxide dismutase in kidney

![Graph showing Superoxide dismutase levels in kidney across different groups.](B)

Figure 5.54: Effect of Ethanolic extract of *Hordeum vulgare* seeds on antioxidant parameter like malondialdehyde (A) and superoxide dismutase (B) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
Figure 5.55: Effect of Ethanol extract of *Hordeum vulgare* seeds on Kidney reduced glutathione (A) and catalase (B) in STZ-nicotinamide induced type II diabetic nephropathy. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Diabetic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated; GP VI = Glibenclamide treated (5 mg/kg). * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05
5.2.8 Fructose induced hyperlipidemia

a) General parameters

Fructose supplementation in drinking water produced hyperlipidemia in rats. Hyperlipidemic control rats showed cardinal sign and symptoms of hyperlipidemia. The hyperlipidemic rats showed significant rise in body weight, food intake and water intake when compared with non-hyperlipidemic control rats. Treatment with EHV 500 significantly reduced body weight (Fig. 5.56A) but failed to produce any significant changes in food intake (Fig. 5.56B) and water intake (Fig. 5.56C) when compared with hyperlipidemic control rats.
Figure 5.56: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Body weight (A), food intake (B) and water intake (C) of fructose induced hyperlipidemic rats. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Hyperlipidemic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
b) Analysis of glucose and insulin

Hyperlipidemic control rats produced a significant hyperglycemia and hyperinsulinemia in rats when compared with non-hyperlipidemic control rats. Treatment with EHV produced a significant reduction in elevated glucose dose dependently when compared with hyperlipidemic control rats (Fig. 5.57A). However, EHV 250 significantly decreased the elevated insulin level in hyperlipidemic animals, while EHV 100 and 500 did not produced significant change in elevated insulin level when compared with hyperlipidemic control rats (Fig. 5.57B).

![Glucose in serum](image1)

![Insulin in serum](image2)

Figure 5.57: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum glucose (A) and insulin (B) of fructose induced hyperlipidemic rats. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Hyperlipidemic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
c) Analysis of lipid profile

Hyperlipidemic control rats showed significantly elevated levels of serum triglyceride, Total-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol, Atherogenic risk and decrease in HDL-cholesterol level when compared with non-hyperlipidemic control rats. Treatment with EHV 100, 250 and 500 produced a significant reduction in level of triglycerides (Fig. 5.58A), Total cholesterol (Fig. 5.58B), VLDL-Cholesterol (Fig. 5.59A) and atherogenic risk (Fig. 5.59C). Serum LDL-Cholesterol level significantly decreased by EHV 500 only (Fig. 5.58C). EHV 500 significantly increased the level of HDL-Cholesterol (Fig. 5.59B) when compared with hyperlipidemic control rats. However, EHV 100 and 250 did not produce any significant effect on HDL-cholesterol and LDL-cholesterol levels when compared with hyperlipidemic rats.
Figure 5.58: Effect of Ethano lic extract of *Hordeum vulgare* seeds on Serum triglyceride (A), cholesterol (B) and LDL-cholesterol (C) of fructose induced hyperlipidemic rats. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Hyperlipidemic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.59: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum VLDL-cholesterol (A), HDL-cholesterol (B) and atherogenic index (C) of fructose induced hyperlipidemic rats. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Hyperlipidemic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
d) Analysis of oxidant-antioxidant parameters

The EHV produced significant effect on antioxidant parameters in hyperlipidemic rats. The hyperlipidemic rats showed significantly increased level of malondialdehyde and decreased concentrations of superoxide dismutase, reduced glutathione and catalase as compared with non-hyperlipidemic rats. The treatment with EHV 500 produced significant reduction in level of malondialdehyde (Fig. 5.60A) and significantly increased concentration of antioxidant enzyme like reduced glutathione (Fig. 5.60B), superoxide dismutase (Fig. 5.61A) and catalase (Fig. 5.61B) in hyperlipidemic rats as compared to hyperlipidemic control rats.

![Graph A: Melondialdehyde (MDA)](A)

![Graph B: Reduced glutathione (GSH)](B)

Figure 5.60: Effect of Ethanolic extract of *Hordeum vulgare* seeds on Kidney malondialdehyde (A) and reduced glutathione (B) of fructose induced hyperlipidemic rats. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Hyperlipidemic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.
Figure 5.61: Effect of Ethanol extract of *Hordeum vulgare* seeds on Kidney Superoxide dismutase (A) and Catalase (B) of fructose induced hyperlipidemic rats. Each bar represents Mean ± S.E.M. Number of animals in each group = 6. GP I = Normal Control; GP II = Hyperlipidemic control; GP III = EHV 100 mg/kg treated; GP IV = EHV 250 mg/kg treated; GP V = EHV 500 mg/kg treated. * Significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.