Study of *Tukhm-e-Kasni* (*Cichorium intybus* L. seed) for steroidal and metabolic effect in albino rats

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Seeds of *Cichorium intybus* L. (*Tukhm-e-Kasni*) are mainly described to be anti-inflammatory, diuretic, tonic; useful in renal asthenia and nephritic syndrome like condition. In the present study 70% ethanol extract of seeds was investigated for its steroidal and metabolic activity in albino rats of either sex in two different tests. In both the tests, the animals were treated with the test drug (150 mg/kg/p.o.) twice a day for three days and were sacrificed subsequently on 4th day. In the test designed for steroidal activity, thymus gland was dissected out and weighed while in the test for metabolic activity, liver was dissected out for glycogen estimation and blood was collected for the estimation of blood sugar, serum protein and serum cholesterol. The test drug reduced the thymus weight significantly (p<0.01) as compared to the plain control and was found to be moderately lesser than the regression produced by hydrocortisone. Further, the study shows that it also induced hyperproteinemia and liver glycogen increasing effect and moderately increased the blood glucose level. The findings suggest that *Tukhm-e-Kasni* possesses marked steroidal and metabolic activity. Steroidal effect may be one of the bases for its use in various renal diseases especially nephrotic syndrome like condition.

Keywords: Steroidal activity, Metabolic activity, *Tukhm-e-Kasni*, *Cichorium intybus* L.

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The seed of *Cichorium intybus* L. (Family:Solanaceae) commonly known as *Tukhm-e-Kasni* is in use in *Unani* system of medicine (*Tibb-e-Unani*) since ancient times to ameliorate various renal diseases. It’s attributed effects in *Unani* literature such as kidney tonic, diuretic¹, anti-inflammatory effect on visceral organs including kidneys²³; useful in cases of as cites and renal asthenia⁴, etc., are considered instrumental for its efficacy in various renal diseases. Ethnobotanical reports suggest almost similar effects. In a recent study it has been shown to possess significant nephroprotective effect against gentamicin induced nephrotoxicity in experimental animals. It also improved a condition which was simulating with the symptoms of nephrotic syndrome². It was hypothesized therefore that the steroidal effect (immunological/anti-inflammatory) may be one of the reasons for its efficacy in such a condition. Therefore, the hydroalcoholic (30:70) extract of *Tukhm-e-Kasni* was studied for steroidal effect by Thymus Regression Test⁵. Further since the steroidal agents induce certain metabolic effects therefore metabolic activity was also studied by observing its effect on liver glycogen, serum glucose, serum protein and serum cholesterol levels⁶.

**Methodology**

**Preparation of ethanol extract**

The seeds of *Cichorium intybus* L. were procured from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh, India. Prof S H Afaq and Dr M Inamuddin (Pharmacognosists), Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh confirmed the identity of the drug. A voucher specimen (No WA/2005/2) has been deposited in the museum of the department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh for future reference. The seeds were dried at room temperature and reduced to coarse powder by grinding. Powdered drug was immersed in 70% ethanol and left for 12 hrs at room

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temperature and then extracted for 6 hrs in soxhlet apparatus at 82±2 °C. 100 gm of powdered drug was extracted in 500 ml of solvent. The filtrate, obtained after filtration of liquid extract through Whatman filter paper was concentrated over a water bath. The yield of the extract was found to be 10% of crude drug (w/w). 70 % ethanol is used because the highest yield percentage was found at this concentration. The extract was reconstituted a fresh in distilled water whenever it was intended to be administrated to the animals.

**Experimental animals**

Wistar Albino rats of either sex weighing 40-50 gm (Thymus regression test) and 100-150 gm (Metabolic test), divided into three groups of six animals each were used. They were maintained on standard diet and water *ad libitum* unless stated otherwise, and housed in clean polypropylene cages at room temperature (25±2°C) with a 12 hrs light: 12 hrs dark cycle. The Institutional Animal Ethics Committee approved the experimental protocol.

**Treatment schedule**

Dose of the test drug for albino rats was calculated by multiplying the human therapeutic dose, described and practiced in Unani Medicine by conversion factor of 7. The dose thus calculated was found to be 150 mg/kg. The test drug suspended in distilled water was administered to the animals intra-gastrically with the help of a gastric cannula twice a day.

**Test for steroidal activity**

The test drug was studied for steroidal effect. Albino rats of either sex, weighing 40-50 gm were divided into 3 groups of 6 animals each having equal distribution of sexes and such that the total weight of animals in various groups were approximately the same. The animals in Group I served as plain control and received 3 ml of distilled water by oral route, twice a day, for 3 days. The animals in Group II serving as standard control were treated with Hydrocortisone 33.33 µg/100 gm, twice a day, for 3 days, by subcutaneous injection. While the animals in Group III served as test group and treated with the hydroalcoholic extract of seeds of *Cichorium intybus* (*Tukhm-e-Kasni*) at a dose of 150 mg/kg, twice a day, oral route. The concentrated extract was reconstituted in suspension form with distilled water (450 mg/3 ml, w/v) and 2% gum *Acacia*, before the administration. On the 4th day all the animals were sacrificed by overdosing of anaesthetic ether, administered by inhalation and the thymus gland was dissected out. The body weight and the weight of the thymus gland were recorded. The results were expressed as mg of thymus gland/100 gm of body weight.

**Test for metabolic activity**

The metabolic effect of the test drug was studied on liver glycogen, serum glucose, serum protein and serum cholesterol in albino rats. Albino rats of either sex, weighing 100-150 gm were divided into 3 groups of 6 animals each having equal distribution of sexes in such that the total weight of animals in various group were approximately the same. They were treated in the same way as in the previous test. On the 4th day all the animals were sacrificed by overdosing of anaesthetic ether, administered by inhalation, and blood sample was collected by cutting the throat for the estimation of blood sugar, serum protein and serum cholesterol, while the liver was dissected out for glycogen estimation.

**Statistical analysis**

The results were given as mean ± S.E.M. Significance was determined by using the student’s *t*-test. *P*-value equal to or less than 0.05 showed significance.

**Results**

**Steroidal effect**

In plain control group the mean thymus weight was found to be 231.40 ± 0.314 mg/100 gm of body weight, while in the standard group treated with hydrocortisone, 33.33 µg/100 gm, it reduced to 146.54 ± 0.355 mg/100 gm of body weight (p<0.001). The weight of thymus gland in the animals treated with the ethanol extract of seeds of *Cichorium intybus* (*Tukhm-e-Kasni*) in a dose of 150 mg/kg, was found to be 161.70 ± 0.497 mg/100 gm (p<0.005). The results are presented in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thymus Weight (mg/100gm) (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Plain control)</td>
<td>231.40 ± 0.314</td>
</tr>
<tr>
<td>Group II (Standard control)</td>
<td>146.54 ± 0.355</td>
</tr>
<tr>
<td>Group III (Test)</td>
<td>161.70 ± 0.497</td>
</tr>
</tbody>
</table>

*n = 6*
Metabolic effect

**Effect of test drug on liver glycogen**

The liver glycogen was found to be 14.12±0.43 mg/gm in plain control group while it increased to 28.89±0.23 mg/gm (p<0.001) in the standard group treated with hydrocortisone, 33.33 µg/100 gm of body weight. In the animals treated with the ethanol extract of test drug it increased to 17.55±0.14 mg/gm (p<0.005).

**Effect of test drug on serum glucose**

Serum glucose was found to be 76.32±0.45 mg/dl in the plain control group while it increased to 114.74±0.86 mg/dl (p<0.001) in standard group treated with hydrocortisone. In the animals treated with the test drug it increased to 85.38±0.92 mg/dl (p<0.05). Glucose level was significantly lower (p<0.01) in test group as compared to standard group.

**Effect of test drug on serum protein**

Serum protein was found to be 5.74±0.49 gm/100 ml of serum in plain control group while in hydrocortisone treated group it amounted to 9.76±0.79 gm/100 ml of serum (p<0.05). In the animals treated with the extract of test drug, it was found to be 6.93±0.57 gm/100 ml of serum showing a significant increase as compared to plain control (p<0.05).

**Effect of test drug on serum cholesterol**

Serum cholesterol was found to be 173.54±0.52 mg/dl in plain control group. It increased to 201.33±0.42 mg/dl (p<0.05) in hydrocortisone treated animals. However, it decreased slightly to 160.67±0.39 mg/dl in the group of animals treated with the test drug (p<0.005). The results of metabolic effects are presented in Table 2.

**Discussion**

The study reveals that the test drug *Cichorium intybus* possesses significant steroidal activity. The thymolytic activity of hydrocortisone and its analogues particularly in immature animals is well documented. In an *in vitro* study it has been shown that basophilic cells normally found in 12 and 13-day embryonic thymus glands disappeared after steroid treatment. Thymus regression effect of steroids was also evident from the findings of the present study where the weight of thymus gland was found to be decreased significantly (p<0.001) under the influence of hydrocortisone. Similarly, the test drug by reducing the weight of thymus gland significantly (p<0.005), which was only moderately lesser than the regression produced by hydrocortisone, indicated having thymolytic and thereby steroidal effect. Since, the steroids have an immunosuppressant effect, which is the basis of their therapeutic application in nephrotic syndrome therefore the efficacy of test drug in nephrotic syndrome like condition for which it has been recommended in *Unani* literature and validated in an experimental study, may be attributed at least partially to its steroidal effect. This finding is also suggestive of its potential to alleviate other diseases where steroids may have a role. Other effects reported to the test drug such as diuretic and anti-inflammatory, etc., may have a direct bearing on nephrotic syndrome and related conditions and/or act as adjuvant to the principal drug. It is interesting to mention that steroids in addition to their immunosuppressive effect also possess anti-inflammatory effect and may modify the body’s immune response to diverse stimuli. Thus, the combined nephroprotective, anti-inflammatory, diuretic and steroid like effect the test drug is attributed with, appears to be in direct commensuration with the physiopathology of nephrotic syndrome. The findings are also suggestive that the diseases with diverse physiopathological appearance can be treated even with a single drug of *Unani* medicine because they commonly have multiple and related, even synergistic effects. This is one of the many advantages that crude drugs have over the isolated compounds.

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**Table 2**—Effect of *Tukhm-e-Kasni* metabolic parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver glycogen (mg/gm) (Mean±S.E.M.)</th>
<th>S. glucose (mg/dl) (Mean±S.E.M.)</th>
<th>S. protein (gm/100 ml) (Mean ± S.E.M.)</th>
<th>S. cholesterol (mg/dl) (Mean±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Plain control)</td>
<td>14.12±0.43</td>
<td>76.32±0.45</td>
<td>5.74±0.49</td>
<td>173.54±2.52</td>
</tr>
<tr>
<td>Group II (Standard control)</td>
<td>28.89±0.23</td>
<td>114.74±0.86</td>
<td>9.76±0.79</td>
<td>201.33±0.242</td>
</tr>
<tr>
<td>Group III (Test drug)</td>
<td>17.55±0.14</td>
<td>85.38±0.92</td>
<td>6.93±0.57</td>
<td>160.67±0.39</td>
</tr>
</tbody>
</table>

n = 6
Steroidal agents play important part in controlling salt and water balance in the body, and regulating carbohydrate, fat, and protein metabolism. They are responsible for certain metabolic effects although when they are used in immunosuppressive and anti-inflammatory therapy their metabolic and other effects are taken as unwanted side effect\textsuperscript{11}. Therefore, different metabolic effects induced by the steroids are mostly not desirable therapeutically. The present study showed that the test drug has significant hyperproteinaemic and liver glycogen increasing effects. It also produced moderate hyperglycaemia which was significantly less than the findings of standard group, but did not alter the cholesterol level (Table 2). These findings are more or less in consonance with steroidal activity\textsuperscript{8}. Thus the metabolic effects produced by \textit{Cichorium intybus} further confirmed that it possessed steroidal effect. Hyperglycaemia and hypercholesterolaemia are not desirable effects of steroids at all whereas hyperproteineemic and glycogenic effects at occasions are used therapeutically. By demonstrating moderate effect on glucose level and not modifying the cholesterol level, the test drug exhibited that it has relatively lesser chances of producing unwanted side effects and is therefore safer than the common steroidal agents. Thus, the findings of the present study have shown that the seed of \textit{Cichorium intybus} possesses marked steroidal and metabolic activity. The steroidal effect may be the basis of its wide therapeutic application in various renal disorders including nephrotic syndrome like condition as described by \textit{Unani} physicians. It has a definite edge over the pure steroids on account of having minimum chances of producing side effects that are common to the steroidal drugs.

**Acknowledgement**

We are grateful to Prof KMY Amin (Pharmacologist), Department of Ilmul Advia, Faculty of Unani medicine, Aligarh Muslim University, Aligarh for guiding us to formulate the study design. We also thank Prof S H Afaq and Dr M Inamuddin (Pharmacognosists) of the same department for helping us to undertake the necessary pharmacognostical studies to confirm the identity of the test drug.

**References**

1. Caius JF, \textit{The Medicinal and poisonous plants of India}, (Scientific publishers, Jodhpur), 2003, 335.