QUALITATIVE SCREENINGS OF PHYTOCHEMICALS AND ANTIHYPERGLYCAEMIC ACTIVITY ON LEAVES OF *URTICA DIOICA* USING STEPTOZOTOCIN INDUCED DIABETIC MICE MODEL

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ABSTRACT

Objective: To study the anti hyperglycaemic effect of aqueous extract of the leaves of *Urtica dioica* streptozotocin (STZ) induced diabetic mice. The leaves extract of *Urtica dioica* doses 100 and 200 mg/kg b.w was administered for 21 days and blood glucose level, serum cholesterol, liver glycogen was estimated. Treatment of the Streptozotocin induced diabetic mice with the leaves extract resulted in significant reduction of blood glucose level (P<.0001), serum cholesterol (P<.01) and increase in liver glycogen (P<.0001). The results suggest that the leaves extract of *Urtica dioica* possess anti hyperglycaemic effect in Streptozotocin induced diabetic mice which justify the traditional use of this plant as ethnomedicine in treatment of diabetes.

Keywords: *Urtica dioica*, Antihyperglycaemic, Diabetes mellitus, Streptozotocin, Oral administration.

1. INTRODUCTION

In India, the indigenous systems of medicine, namely Ayurvedic, Siddha and Unani, have been in existence for several centuries. These traditional systems of medicine together with Homoeopathy and Folklore medicine continue to play a significant role in the health care system of the population. Besides the demands for medicinal plants made by these systems as their raw material, the demand for medicinal plants made by the modern pharmaceutical
industries has also increased manifold. Thus, medicinal plants constitute a group of industrially important crops which bring appreciable income to the country by way of export (Ganesan et al., 2006).

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogenous group of disorders having hyperglycaemia which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response[1]. Apart from currently available therapeutic options for Diabetes like oral hypoglycaemic agents and insulin which have limitation of their own, many herbal medicines have been recommended for the treatment of Diabetes[2]. A variety of ingredients present in medicinal plants are thought to act on variety of targets by various modes and mechanisms. They have a potential to impart therapeutic effect in complicated disorders like Diabetes and its complications[3]. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand of antidiabetic medicinal plant which has comparatively less side effects. Indian traditional medicines belong to one of the richest medicinal systems are among those available in the world. Especially North Eastern part of India is blessed with a very rich biodiversity with a rich wealth of traditional knowledge which is yet to be explored. So more and more research is required to explore the traditional knowledge of this region. According to the recommendation of the WHO expert committee on Diabetes mellitus (WHO, 1980), an investigation of hypoglycaemic agents of plant origin used in traditional medicine seems important.

_Urtica dioica_ is found in the sub tropical areas of Himalaya reasions, [4]. _Urtica dioica_ is a medicinal herb which belongs to Urticaceae family. It is known as Vasaka in Hindi. An evergreen shrub upto 2.4 m high, branchlets quadrangular, leaves are 13-35 cm long, oblanceolate, elliptic oblong, acute or acuminate, entire. Leaves are in terminal elongated, thyrsoid panicles, upto 30cm long. Capsule is 3.8 cm long, linear clavate. In early spring the plant becomes showy with its dense cylindrical spikes of brick red velvety leaves. Calyx lobe is 6.8 mm, bristly haired. Bracts are 6 to 12 mm long. Seeds are disc like. Leaves occurs in the month of February to April[5]. The leaves are reported to contain diterpene lactone, Phlogantholide A. A decoction of leaves is also beneficial in liver and spleen diseases[4]. Jaintia tribe of Meghalaya uses fruit and leaf ash of _Urtica dioica_ and use it to treat fever[6]. Ethanolic extract of _Urtica dioica_ has analgesic activity on experimental mice[7]. _Urtica dioica_ has antimicrobial activity also[8]. The generation of free radicals has been implicated in
the causation of several diseases of known and unknown etiologies such as Rheumatoid Arthritis, Cancer, Diabetes etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. *Urtica dioica* has prominent free radical scavenging property so it may prove as a very good medicinal herb\(^9\).

## 2. MATERIALS AND METHOD

### 2.1-A: Collection and Identification of Crude drugs

The common and locally available Ayurvedic crude drugs were collected from Himalaya commercial markets. All samples were identified through comparison with standard references.

### 2.1- B: Chemicals

Streptozotocin and Glibenclamide was purchased from Sigma Chemical Co, St Louis, MO, USA. All other chemicals and reagents used were of analytical grade.

### 2.2. Plant material

The leaves of *Urtica dioica* were collected from local market in June 2013 and herbarium was prepared. The herbarium was identified for authenticity by the experts of Dept of Botany, Sagar Institute of Research, Technology & Science- Pharmacy, Bhopal, M.P. The leaves were thoroughly washed and shade dried.

### 2.3. Preparation of Plant extract

After shade drying the dried leaves were powdered in mixture grinder. The powdered leaves were macerated with distilled water for 72 hrs at room temperature with occasional stirring. It was then filtered through Whatman filter paper. The filtrate was air dried and stored in refrigerator for further use as AWAE (*Urtica dioica* aqueous extract). The yield of the extract was 10% (w/w). During experiment the crude extract was diluted with distilled water just before administration to animals.

### 2.4. Phytochemical screening

**Qualitative screenings of Phytochemicals**

The qualitative screenings of powdered crude drugs for their active ingredients were carried out using the following standard procedures (Trease and Evans, 1983; Indian Pharmacopeias, 1996; Mukherjee,2002; Horborne, 2005).
Test for Alkaloids
Extract 2g of powdered drug by warming for 2 min. with 20ml 1% sulphuric acid in a 50ml conical flask on a water bath, with intermittent shaking, centrifuge; pipette off the supernatant into a small conical flask. Make an initial test for alkaloids by adding to 0.1 ml extract in a semi-micro tube, one drop of Meyer’s reagent. It gives a cream precipitate with alkaloids.

Preparation of Mayer’s reagent
It is prepared by dissolving 1.36g of mercuric chloride in 60 ml distilled water (A) and 5g of potassium iodide in 60ml of distilled water (B). A and B are mixed together and the volume adjusted to 100ml with water.

Test for Essential oil / Volatile oil
Test 1.
Crush a small sample of the crude drug between the thumb and forefinger, and examine for the presence of an odour.
Observation: Drug containing volatile oils have a strong odour.

Test 2.
Extract 1g of the powdered drug by warming with 10ml petroleum spirit (boiling point range 40-60 C.) in a boiling tube heated on a water bath. Do not let the solvent boil dry. Filter the mixture into an evaporating dish and concentrate the filtrate to about 1ml on a water bath. Using a pipette apply one drop of the extract to a filter paper. Expose the paper to a current of warm air and note the occurrence of any translucent area. If this observed, then oils are present.

Observation 1.
Place the paper in an oven at 105 C for 15min. and if the translucent spot can still be observed after that time, then a fixed oil is present.

Observation 2.
The presence of a volatile oil is detected by the disappearance or diminution of the translucent area.

Test for Flavonoids (flavone)
Test 1.
Prepare an aqueous filtrate of powdered drug, and take a portion of filtrate in a test tube, add
5 ml of dilute ammonia followed by add few drops of concentrated sulphuric acid. A yellow coloration appears. Upon further standing, the yellow coloration disappears.

**Test 2.**
Take a small amount of powdered drug in a test tube, add 10 ml ethyl acetate and heat it over a steam bath for 3min. then filter the mixture, take 4 ml of the filtrate with 1ml of dilute ammonia solution. Observe the formation of yellow colouration. It is the indication of flavanoids compounds of drug.

**Test for Glycosides**

**Test 1**
Extract 200 mg of the sample by warming in a test tube with 5ml of dilute (10%) sulphuric acid (Test with PH paper) on a water bath at 100C for 2min. centrifuge or filter, pipette off the supernatant or filtrate. Neutralize the acid extract with 5% solution of NaOH (Noting the volume of NaOH added). Add 0.1ml of Fehling’s solution ‘A’ and then Fehling’s solution ‘B’ until alkaline (Test with PH paper) and heat on the water bath for 2min. Note the quantity of red precipitate formed and compare with that formed in Test 2.

**Test 2**
Extract 200mg of the sample using 5ml of water instead of sulphuric acid. After boiling add a volume of water equivalent to the volume of NaOH used in Test 1. Add 0.1ml of Fehling’s solution A and then Feling’s solution B until alkaline (test with PH paper) and heat on the water bath for 2min. and note the quantity of red precipitate formed (Test.2.)

Compare the quantity of precipitate formed in Test 2 with that formed in Test 1. If the precipitate in Test 1 is greater than that in Test 2, then glycosides may be present, since Test 2 represents the amount of free reducing sugars already present in the crude drug, whereas Test 1 represents free reducing sugars plus those released on acid hydrolysis of any glycosides in the crude drug.

**Tests for Potassium Salts**
Dissolve 0.1g of substance being examined in 2 ml of water. Heat the solution with 1ml of sodium carbonate solution (10.6% w/v), no precipitate is formed. Add 0.05 ml of sodium
sulphite solution (10%), no precipitate is formed, cool in ice, add 2 ml of a 15% w/v solution of tartaric acid and allow to stand, a white crystalline precipitate is produced.

**Test for Saponin**
Take 2g of the powdered sample and boil with 20 ml of distilled water in a water bath and filter it, 10 ml of filtrate is mix with 5 ml of distilled water and shake vigorously for a stable persistent froth. To this froth mix 3 drops of olive oil and shake vigorously, then observe for the formation of emulsion.

**Tests for Starch**
Take 1g of dry powder in 50 ml of water boil for one minute and cool, a thin and cloudy mucilage is produced, which gives thick and more transparent mucilage. To 10 ml of the mucilage add 0.05 ml of 0.01M Iodine, a dark blue colour is produced, which disappears on heating and reappears on cooling.

**Test for Tannins**
Take 0.5g of the dried powdered sample in 20ml of water, boil on a water bath and filter it in a test tube. Add few drops of 0.1% ferric chloride and observe for brownish green or a blue-black colouration.

**Test for Terpenoids (Salkowski test)**
Take five ml of extract, mixed with 2 ml of chloroform, and concentrated H2SO4 (3ml) is added to form a layer. A reddish brown colouration on the inner face is formed. It is indicates the presence of terpenoids.

**Test for Vitamin C or Ascorbic Acid**
To 2ml of 2% w/v solution, add 2ml of water, 0.1g of sodium bicarbonate and about 20mg of ferrous sulphate, shake and allow stand; a deep violet colour is produced. Add 5ml of 1M sulphuric acid, the colour disappears.

Phytochemical screening of the crude plant material was carried on using standard protocols for detection of flavonoid, phenol, tannin, saponin, steroid, alkaloid, carbohydrate.\[21\],\[22\],\[23\],\[24\].
2.5. Experimental Animals
Healthy adult albino mice of both sexes (20-25 g) in house bred at the Animal house of Sagar Institute of Research, Technology & Science- Pharmacy, Bhopal, M.P. India were used for the study. Mice were housed in polypropylene cages lined with husk in standard environmental conditions and 12:12 light:dark cycle. The animals were fed on a standard pellet diet *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

2.6. Experimental Design
Antidiabetic activity of *Urtica dioica* aqueous extract was assessed in normal, glucose loaded hyperglycaemic and streptozotocin induced diabetic mice. In all studies, the animals were fasted overnight for 16h with free access to water throughout the duration of the experiment.

2.7. Evaluation of extract on normal healthy mice
At the end of the fasting period taken as zero time (0 h), blood was withdrawn from the tail vein. Serum was separated by centrifugation and glucose was estimated. The animals were randomly divided into four groups of six animals each. Group 1 served as control and received only distilled water. Group II, III and IV received *Urtica dioica* orally at the dose of 50, 100, 200 mg/kg. Blood glucose levels were determined in 1, 2, 3h following treatment.

2.8. Evaluation of extract in Oral glucose tolerance test
Healthy mice were divided into four groups of six animals each: Group I served as control received only vehicle (distilled water) and Groups II, III and IV received *Urtica dioica* orally at the dose level of 50, 100, 200 mg/kg, respectively. All the animals were given glucose (2g/kg) 60 min after dosing. Blood samples were collected from tail vein just prior to (0h) and at 30, 60, 90 and 120 min after glucose loading and blood glucose levels were estimated.

2.9. Evaluation of extract in streptozotocin induced diabetic mice
Experimental diabetes was induced by single intraperitoneal injection of 55mg/kg of Streptozotocin (STZ) freshly dissolved in distilled water. Control animals received only distilled water. After 48 hrs of Streptozotocin injection animals with fasting blood glucose above 200mg/dl were considered as diabetic and included in the study. The animals were randomly assigned into five groups of six animals each and received the following treatments: Group I: Normal control + distilled water, Group II: Diabetic control + distilled
water, Group III: Diabetic + *Urtica dioica* (100mg/kg), Group IV: Diabetic + *Urtica dioica* (200mg/kg), Group V: Diabetic+ Glibenclamide (10mg/kg).

The freshly prepared solutions were orally administered daily for 21 days. Body weights and blood glucose analysis was done weekly on overnight fasted animals. At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation. Liver was dissected out, immediately rinsed in ice cold saline and stored for further biochemical analysis.

2.10. Biochemical analysis

Serum glucose analysis was done by GOD-POD method using Glucose Estimation kit (Crest Biosystems). Serum Cholesterol was estimated spectrophotometrically (CHOP-PAP method, Crest Biosystems). Liver glycogen was estimated by the method of Seifter Sam *et al* (1950)[18].

2.11. Acute oral toxicity study

Acute oral toxicity of *Urtica dioica* was performed on Swiss albino mice, according to OECD Guidelines 423. Two groups of three animals in each were used for the study. Group I received distilled water. Group II received oral dose of 1000mg/kg for 3 days. The animals were observed for gross behavioral, neural, autonomic and toxic effects at short intervals of time for 24 hrs and then daily for 7 days. A food consumption and body weight was monitored daily.

2.12. Statistical analysis

All results were expressed as mean ± SEM. The significance of the difference between the means of test and control studies was established by student’s t-test. P value less than 0.01,.001,.0001 were considered significant.

3. RESULTS

Identification of plants with botanical verifications is essential as contamination due to misidentification of plant species or parts is common. Characterizing compound or biomarker is identified from the plant part to assure the identity and quality of the preparation, this need not be
responsible for the therapeutic activity. Details including various names (binomial, vernaculars, etc.) with collection conditions, and part to be used should be documented to ensure proper identification (WHO, 2001).

The therapeutic potentials of plant and animal origins have been used from ancient times by a simple process without the isolation of pure compounds (i.e. in the form of crude drugs or the galenicals prepared from them). The pharmacological action of crude drug is determined by the nature of its constituents (Mukherjee, 2002), such as alkaloids, terpenoids, flavonoids, glycosides, saponins, tannins, etc.

A test requirement for foreign matter would ensure the extent of contamination of extraneous matters, such as filth and other parts of botanicals not covered by the definition of the herbal drug. Since sand and soil are predicable contaminations of botanicals, test requirements for total ash, water-soluble ash, acid insoluble ash, residue on ignition and sulphated ash would be expected to limit such contaminants (Handa, 2005).

3.2. Effect of *Urtica dioica* aqueous extract on normoglycaemic mice

Results of the effect of graded doses of *Urtica dioica* on blood glucose level in normal healthy mice are presented in Table 4. *Urtica dioica* produced peak hypoglycaemia at 2h. Dose dependent blood glucose reduction was observed in animals treated with 50, 100, 200 mg/kg. *Urtica dioica* at dose 200mg/kg showed significant reduction in blood glucose (P<.001) when compared to control. Blood glucose levels were restored in all treatment groups in 3h.

3.3. Effect of *Urtica dioica* aqueous extract on oral glucose tolerance in normal mice:

*Urtica dioica* when administered 60 min prior to glucose loading produced significant reduction in the rise in blood glucose levels at 60 min after glucose administration which is shown in Table 5. Dose dependent blood glucose reduction was observed in animals treated with 50, 100, 200 mg/kg. All the doses showed significant reduction in blood glucose (P<.001) when compared to control.

3.4. Effect of *Urtica dioica* aqueous extract on fasting blood glucose and body weight in STZ induced diabetic mice

The effect of repeated oral administration of *Urtica dioica* on blood glucose levels in Streptozotocin induced diabetic mice and body weight is given in Table 6 and Table 7.
Urtica dioica administered in two different doses to Streptozotocin treated diabetic mice showed significant reduction of blood glucose levels which was related to dose and duration of the treatment. Maximum reduction was observed on day 21. Urtica dioica both doses 200mg/kg, 100mg/kg exhibited significant glucose lowering effect in diabetic mice (P<.0001) as compared to the control. Streptozotocin produced significant loss of body weight as compared to normal animals during the study. Diabetic control continued to lose weight till the end of the study while Urtica dioica treated group at all the two doses showed improvement in body weight compared to diabetic control.

3.5. Effect of Urtica dioica aqueous extract on serum cholesterol and Liver glycogen in STZ induced diabetic mice

Urtica dioica treated group showed reduction in serum cholesterol compared to the diabetic control which is shown in Table 8. Urtica dioica both the doses 200mg/kg, 100mg/kg were effective in reducing the cholesterol levels (P<.01). Glycogen content in liver decreased in diabetic control compared to normal control. Administration of Urtica dioica at the doses of 100 and 200 mg/kg for 21 days resulted in significant increase in the glycogen levels in liver (P<.0001) which is shown in Table 8.

3.6. Acute Oral Toxicity Study

Urtica dioica showed no mortality or behavioural change upto 1000mg/kg in the animals.

Table 1: Crude Drug and its Description

<table>
<thead>
<tr>
<th>Name of the Sample</th>
<th>Local Name</th>
<th>Appearance</th>
<th>Organoleptic Test</th>
<th>Location of Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urtica dioica</td>
<td>Talispatra Leaves</td>
<td>Dried with small stem pieces</td>
<td>Mild astringent with Characteristic odour</td>
<td>Himalaya Region</td>
</tr>
</tbody>
</table>

Table 2: Purity and Strength of Crude Drugs.

<table>
<thead>
<tr>
<th>(Name of the Sample Binomial)</th>
<th>PH*</th>
<th>Loss On Drying* (%)</th>
<th>Water Soluble Extractive* (%)</th>
<th>Ethanol Soluble Extractive* (%)</th>
<th>Total Ash* (%)</th>
<th>Acid Insoluble Ash* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urtica dioica</td>
<td>5.37</td>
<td>08</td>
<td>34</td>
<td>16.8</td>
<td>06</td>
<td>01</td>
</tr>
</tbody>
</table>

* = Each value is a mean of Triplicates
Table 3: Screening of Active Principles of Crude Drugs.

Phytochemical screening of leaves of *Urtica dioica* showed the presence of following:

<table>
<thead>
<tr>
<th>Name of the Sample (Binomial)</th>
<th>Active principle</th>
<th>Qualitative Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Urtica dioica</em></td>
<td>Tannins, Essential oil</td>
<td>Alk1  Es/Vo  Fla  Gly  Pos  Sap  Sta  Tan  Ter  Vit.C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+  +  -  -  -  -  +  -  -  -</td>
</tr>
</tbody>
</table>


Table 4: EFFECT OF *URTICA DIOICA* AQUEOUS EXTRACT IN NORMOGLYCAEMIC MICE (Mean±SEM)(n=6)

<table>
<thead>
<tr>
<th>SL NO</th>
<th>GROUPS</th>
<th>DOSES(mg/kg)</th>
<th>BLOOD GLUCOSE LEVELS(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr 1hr 2hr 3hr</td>
<td>1hr 2hr 3hr</td>
</tr>
<tr>
<td>1.</td>
<td>I(control)</td>
<td>Distilled water</td>
<td>71±.58 74.6±.33 71±.58</td>
</tr>
<tr>
<td>2.</td>
<td>II</td>
<td>50</td>
<td>74.5±.5 70.3±.33 64.5±.33</td>
</tr>
<tr>
<td>3.</td>
<td>III</td>
<td>100</td>
<td>74.5±.5 70.3±.33 62.6±.33</td>
</tr>
<tr>
<td>4.</td>
<td>IV</td>
<td>200</td>
<td>80.5±.5 74.6±.33 62.6±.33</td>
</tr>
</tbody>
</table>

*P<.01 when compared with corresponding values of control group

*P<.001 when compared with corresponding values of control group

Table 5: EFFECT OF *URTICA DIOICA* ON ORAL GLUCOSE TOLERANCE IN NORMAL MICE (Mean±SEM)(n=6)

<table>
<thead>
<tr>
<th>SL NO</th>
<th>GROUPS</th>
<th>DOSES(Mg/kg)</th>
<th>BLOOD GLUCOSE LEVELS(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0hr 30min 60min 90min 120min</td>
<td>30min 60min 90min 120min</td>
</tr>
<tr>
<td>1.</td>
<td>I(control)</td>
<td>Distilled water</td>
<td>80.5±.5 140.3±.33 170.3±.33</td>
</tr>
<tr>
<td>2.</td>
<td>II</td>
<td>50</td>
<td>74.5±.5 134.6±.33 140.3±.33</td>
</tr>
<tr>
<td>3.</td>
<td>III</td>
<td>100</td>
<td>74.5±.5 103.3±.33 130.3±.33</td>
</tr>
<tr>
<td>4.</td>
<td>IV</td>
<td>200</td>
<td>83.5±.5 124.3±.33 134.3±.33</td>
</tr>
</tbody>
</table>

*P<.001 when compared with corresponding values of control group

*P<.001 when compared with corresponding values of control group
Table 6: EFFECT OF *URTICA DIOICA*ON BLOOD GLUCOSE IN STZ INDUCED DIABETIC MICE (Mean±SEM)(n=6)

<table>
<thead>
<tr>
<th>ANIMAL GROUPING</th>
<th>BLOOD GLUCOSE LEVELS(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.7 ±5.64</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>209.5±8.35</td>
</tr>
<tr>
<td>Treated 100mg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>204.06±8.24</td>
</tr>
<tr>
<td>Treated 200mg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>207.6±8.31</td>
</tr>
<tr>
<td>Glibenclamide(10mg/kg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>207.6±8.31</td>
</tr>
</tbody>
</table>

a P<.0001 compared to diabetic control
b p<.0001 compared to day 1of same group

Table 7: EFFECT OF *URTICA DIOICA* ON BODY WEIGHT OF STZ INDUCED DIABETIC MICE

<table>
<thead>
<tr>
<th>ANIMAL GROUPING</th>
<th>BODY WEIGHT (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.06±2.88</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.06±2.88</td>
</tr>
<tr>
<td>Treated 100mg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.6±2.91</td>
</tr>
<tr>
<td>Treated200mg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.2±2.94</td>
</tr>
<tr>
<td>Glibenclamide(10mg/kg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.8±2.97</td>
</tr>
</tbody>
</table>

Table 8: EFFECT OF *URTICA DIOICA* ON SERUM CHOLESTEROL AND LIVER GLYCOGEN IN STZ INDUCED DIABETIC MICE

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum cholesterol(mg/dl)</th>
<th>Liver Glycogen(mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.6±.33</td>
<td>38.5±.35</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>82.4± 3.4207</td>
<td>11.86±3.38</td>
</tr>
<tr>
<td>Treated 100mg/kg</td>
<td>55.6± .50</td>
<td>29.6±29</td>
</tr>
<tr>
<td>Treated 200mg/kg</td>
<td>53.2± 1.41</td>
<td>30.7±87</td>
</tr>
<tr>
<td>Glibenclamide(10mg/kg)</td>
<td>48.8± 2.83</td>
<td>31.6±27</td>
</tr>
</tbody>
</table>

a P<.001 Compared to normal control
b P<.01 Compared to diabetic Control
c P<.0001 compared to the corresponding values of normal control
d P<.0001 compared to the corresponding values of diabetic control
4. DISCUSSION
The study was undertaken to evaluate the hypoglycaemic activity of *Urtica dioica* normal, glucose loaded hyperglycaemic and streptozotocin induced diabetic mice. In normoglycaemic mice *Urtica dioica* showed dose dependent hypoglycaemic effect in 2 h. From OGTT it could be concluded that dose 200mg/kg showed maximum improvement in glucose tolerance.

Streptozotocin significantly induced hyperglycaemia. Oral administration of *Urtica dioica* for 21 days caused a significant decrease in blood glucose levels. The possible mechanism by which *Urtica dioica* mediated its antidiabetic effect could be by improvement of pancreatic secretion of insulin from existing β cells of islets. The hypoglycaemic effect of *Urtica dioica* was compared with Glibenclamide, a standard hypoglycaemic drug. From the present study it may be suggested that the mechanism of action may *Urtica dioica* be similar to glibenclamide action. So oral administration of *Urtica dioica* has prominent hypoglycaemic effect.

Hypercholesteremia is one of the primary factor involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes$^{[19]}$. *Urtica dioica* significantly reduced serum cholesterol in STZ diabetic mice. Thus it is reasonable to conclude that *Urtica dioica* could modulate blood cholesterol abnormalities.

Diabetes mellitus impairs the normal capacity of the liver to synthesis glycogen. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and its activation appears to be defective in diabetes$^{[20]}$. Decrease in hepatic glycogen was observed in this study. Treatment with *Urtica dioica* (100 and 200mg/kg) for 21 days significantly increased liver glycogen indicating that the defective glycogen storage of the diabetic state was partially corrected by the extract.

Thus the significant antidiabetic effect of *Urtica dioica* could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone can impart therapeutic effect. From this study we can conclude that aqueous extract of *Urtica dioica* leaves has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetes. Further studies are necessary to elucidate in detail the mechanism of action of the medicinal plant at the cellular and molecular levels. The studies on the effect of *Urtica dioica* aqueous extract on lipid profiles and liver enzymes in Streptozotocin induced diabetic mice is going on in our laboratory.
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