INTRODUCTION: The present study was conducted to identify the phytoconstituents present in different extracts of Sargassum wightii and to assess the toxicity of its ethanol extract.

METHODS: Successive solvent extraction and total ethanol extraction of Sargassum wightii were performed and preliminary phytochemical screening was carried out. Acute toxicity study of ethanol extract of Sargassum wightii (ESW) was carried out. Subchronic toxicity study of ESW was carried out with the doses of 100, 200, 400 mg/kg. The animals were observed for changes in body weight, food and water intake. At the end of study, relative weight of vital organs were noted followed by histopathological examinations. Various hematological, biochemical estimations were also carried out.

RESULTS: Phytochemical screening of Sargassum wightii revealed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds and tannins. ESW did not induce any mortality or pre-terminal death in the acute toxicity study. There were no significant difference in body weight, relative weight of vital organs (except brain), food and water intake compared to the control group. Histopathological examination showed normal architecture suggesting absence of pathological lesions. Hematological and biochemical parameters were also found to be comparable to the control group except reduction in glucose and cholesterol level which is postulated to be beneficial.

DISCUSSION AND CONCLUSION: Presence of various phytoconstituents in Sargassum wightii is evident that it could be a potential source for treating different ailments. No significant toxic effects were observed with the treatment of ESW. Thus, it is proposed to be safe and can be recommended for long term treatment.

Keywords: Sargassum wightii, phytochemical screening, acute toxicity, subchronic toxicity

INTRODUCTION

Sargassum is one of the significant genera of brown marine algae belonging to Sargassaceae family. The well-known bioactive compounds of Sargassum include meroterpenoids, phlorotannins, fucoidans, sterols and glycolipids. It is reported to possess antioxidant, hypolipidemic, hypoglycaemic, neuroprotective, antimicrobial, anticancer, anti-inflammatory, anticoagulant, antimelanogenic and hepatoprotective activities.1-9

Sargassum wightii Greville (S. wightii) is an abundant marine brown algae commonly found in the shorelines of India. It is dark-brown in colour, 21-40 cm in height, richly branched, midrib is spherical to ellipsoidal which is 5-8 mm long and 2-4 mm wide.10 It is a macroscopic, multicellular, photosynthetic, non-vascular, pelagic marine species rich in sulphated
polysaccharides that manifest potent free radical scavenging and antioxidant effects, hypolipidemic and anti-inflammatory effect.\textsuperscript{11-13} Fucoidan is known to be one of the well-known components of \textit{S. wightii} with diverse biological activities including anti-inflammatory, anticancer, antimicrobial, \(\alpha\)-\(D\)-glucosidase inhibitory activity.\textsuperscript{14} The present study was designed to screen the phytoconstituents present in different extracts of \textit{S. wightii} and to assess the toxicity of total ethanol extract of \textit{S. wightii}. Ethanol extract was chosen for toxicity study as it is known to possess various potent bioactive phytoconstituents compared to other solvent extracts and also because of interest in further pharmacological studies with ethanol extract of \textit{S. wightii}.

\textbf{MATERIALS AND METHODS}

\textbf{i) Study Material}

The fresh seaweed of \textit{S. wightii} was collected from coastal regions of Rameshwaram, Tamil Nadu, India during October, 2015. It was authenticated by Dr. Yoganarasimhan, Taxonomist and a voucher specimen (#52) was prepared as per the guidelines and deposited at the Herbarium of Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bangalore, for future reference.

During collection, the seaweeds were washed thoroughly with sea water to get rid of any superfluous matter such as sand particles, salt, epiphytes or any other foreign materials. Later, the seaweeds were thoroughly washed with running tap water and then distilled water. It was shade dried at room temperature, powdered and then packed in airtight containers. The study material was stored in refrigerator for further study. The macroscopic analysis including size, shape, color, base, margin of \textit{S. wightii} was carried out.\textsuperscript{4}

\textbf{ii) Extraction}

\textbf{a) Successive solvent extraction:}
Successive solvent extraction of *S. wightii* was carried out using a Soxhlet apparatus. The solvent order was according to their polarity such as petroleum ether, toluene, chloroform, 95% ethanol, cold maceration. The extract with each solvent was filtered and dried to concentrate. The color and consistency of the extracts were noted and the percentage yield was calculated.

**b) Total alcohol extraction:**

The coarsely powdered plant material was defatted using petroleum ether (60-80 °C) and extracted using 95% v/v ethanol in a Soxhelet apparatus. The extract was filtered, evaporated and accurate weight of the extract was taken. The color and consistency of the extracts were noted and percentage yield was calculated.

**iii) Phytochemical Screening**

Preliminary phytochemical screening was carried out for the extracts obtained from successive solvent extraction and total alcohol extraction according to standard procedures.\(^{15}\)

**iv) Toxicity Studies**

**a) Experimental Animals**

All the animals used for this study were obtained from the Animal house facility, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences (Registration number No: 220/PO/ReBi/S/2000/CPCSEA/ 02.05.2016). Acute toxicity was carried out on three female Wistar rats. For subchronic toxicity study, Wistar rats of either sex weighing between 195 g – 235 g were used in present study. They were divided into four groups, each group consisting of six animals.
The animals were housed in polypropylene cages. The temperature in the experimental animal room was maintained as 22°C (±3°C), with the relative humidity 50-60% and artificial lighting, the sequence being 12 hours light, 12 hours dark. Each animal in the cage were marked in the tail with methylene blue dye for appropriate identification. Food but not water was withheld overnight for experimental animals. Experimental procedures were conducted in accordance with the guidelines provided by Institutional Animal Ethics Committee and prior approval was obtained with the approval no. MSRFPH/PFP-59/2015.

b) Acute toxicity study

Acute toxicity study was designed as per the OECD Guidelines 423. S. wightii being a traditional medicine, with no reports of mortality even in large doses, limit test was carried out. Single oral dose (2000 mg/kg) of ethanol extract of S. wightii (ESW) was given to three female Wistar rats.

After dosing, for the first 30 minutes, animals were monitored individually. They were given special attention for the first 4 hours for any toxic signs. The observation was extended to first 24 hours and daily thereafter for a total of 14 days. The animals were observed individually for any toxic signs or pre-terminal deaths and recorded if any. Once in a week, individual body weight was monitored for all the animals to find out any drastic changes. The colour and consistency of faeces, changes in fur and skin, mucous membranes (nasal) and eyes of the animal were observed on a weekly basis.

Physical observation such as changes in circulatory (heart rate), respiratory (rate), autonomic (piloerection, lacrimation, salivation, urinary incontinence and defecation) and central nervous system (drowsiness, ptosis, gait, eye prominence, eyelid closure, convulsions, biting, straub’s test, motor in-coordination, writhing, stereotypy, aggression, righting reflex,
pinnal reflex, and corneal reflex, tremors and convulsions) were monitored and recorded if any.

c) **Sub-chronic toxicity**

Sub-chronic toxicity study was carried out as per OECD guidelines 407. Animals were divided into four groups, each group consisting of six animals. Grouping of animals was as following:

**Group I** – Normal control vehicle treated 1% sodium CMC

**Group II** – Ethanol extract of *S. wightii* 100 mg/Kg (Low dose)

**Group III** – Ethanol extract of *S. wightii* 200 mg/Kg (Medium dose)

**Group IV** – Ethanol extract of *S. wightii* 400 mg/Kg (High dose)

Group I received 1% sodium CMC, Group II – IV received ESW in the dose of 100, 200 and 400 mg/kg orally once daily for 28 days. Doses were selected depending on the results obtained from acute toxicity study. Dosing time was maintained to be constant to minimize any biological variation among animals.

At the end of the study, animals were anesthetized and blood sample was collected through retro orbital plexus into non-heparinized tubes for biochemical and heparinized tubes for hematological parameters. Biochemical analysis was carried out to explore major toxic effects in tissues, specifically on liver and kidney. Biochemical investigations included sodium, potassium, glucose, total protein and albumin, urea, creatinine, total cholesterol, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST). Hematological parameters such as hemoglobin content, total leucocyte count, erythrocyte count and platelet count were estimated. Urine analysis was carried out during the last week of the study which included timed urine volume, and pH.
All animals in the study were subjected to a complete, detailed gross necropsy. It included thorough examination of the external surface of the body, all orifices, thoracic, cranial and abdominal cavities. The vital organs such as liver, lungs, spleen, brain, kidneys, heart was isolated and adherent tissue was cleared. The wet weight of organs was taken immediately after dissection to avoid drying. Individual organs were also examined macroscopically for any gross lesions in all the animals. The following tissues were preserved in the fixation medium for histopathological examination: brain, stomach, small intestines, liver, kidneys, spleen, heart, lungs, bone, testes, ovary, and pancreas.

v) Statistical analysis

Data were expressed as mean ± SEM (n=6). Significant difference between groups were determined using One-Way ANOVA followed by Tukey's Multiple Comparison test. P < 0.05 was considered as significant.

RESULTS

Macroscopic characters of *S. wightii* leaf were as follows: 4 to 8 cm length, linear to ovale in shape, dark brown in colour, with tapering base and entire margin. The colour, consistency and percentage yield of various extracts are presented in Table 1. The color of extracts was varying from dark green, greenish brown to brown. Consistency was mostly semisolid except the extract of cold maceration, which was solid. Percentage yield of extract ranged from 0.69 – 2.98. The highest yield was from cold maceration (2.98%) and the least from toluene extraction (0.69%). Phytochemical screening revealed the presence of alkaloids, phenolic compounds, carbohydrates, glycosides, and tannins.

**Acute toxicity study**
ESW did not induce any mortality or pre-terminal death. No changes were observed in salivation, lacrimation, perspiration, piloerection, micturition, and defecation. The animals were observed for ptosis, drowsiness, stereotypy, aggression, tremors, convulsion, Straub’s test, motor incoordination, writhing and no abnormalities were observed in all the treated animals. Gait, righting reflex and corneal reflex were found to be normal. Skin, fur, eyes, and body weight of animals were found to be normal. Tremors, lethargy, diarrhoea and coma were not observed throughout the study.

**Sub chronic toxicity study**

Body weight changes were documented on a weekly basis and the results are presented in Table 1. The body weight of animals treated with extract 100, 200 and 400 mg/kg had no significant changes and were comparable with the control group. The results of sub chronic toxicity study on food and water intake is presented in Tables 2 and 3 respectively. Food as well as water intake were monitored in all the groups on a daily basis and the results are presented on a weekly basis. There were no significant changes in food as well as water intake between the control and extract treated groups.

The results of biochemical analysis are reported in table 4. There were no significant changes in parameters such as albumin, total protein, urea, creatinine, sodium, potassium at the end of 28 days treatment. AST and ALT did not show any significant difference in all the extract treated groups and was found to be comparable with the control group.

There was a statistically significant decrease in blood glucose levels with the extract treated groups. Cholesterol level was found to be significantly reduced in the extract treated animals at doses 100 and 200 mg/kg whereas 400 mg/kg did not show any significant differences.
Hematological parameters were estimated at the end of 28 days and the results are presented in table 5. There were slight variations but no significant changes in the level of Red Blood Cell (RBC), White Blood Cell (WBC), platelets, hemoglobin in all the extract treated groups when compared to the normal group. The results of urine analysis (Table 6) were found to be comparable with that of control group.

Gross anatomy of every organ was examined macroscopically by direct observation. The macroscopical architecture of organs was found to be normal with all the treatment groups and there were no signs of abnormalities. The organ weight of control and extract treated animals were noted at the end of the study (Table 7). There were no significant changes in the weight of vital organs including heart, kidney, liver, spleen, lungs whereas there was a slight increase in the weight of brain with the dose of 400 mg/kg.

Histopathological evaluation was conducted on various organs such as kidney, lungs, testes, ovary, bone, stomach, brain, pancreas, spleen, intestine, liver, heart. Testes showed seminiferous tubules with normal spermatogenesis in low dose treated groups. Increase in spermatogenesis with normal architecture was observed in medium and high dose treated groups (Fig. 1a, 1b, 1c, 1d). With low dose, the glomeruli appeared normal with mild tubular epithelial damage. Medium dose treated animals showed mild infiltrations in the glomeruli with tubular epithelial cell damage and the damages were comparable to the control group (Fig. 2a, 2b, 2c, 2d). All the groups showed functionally efficient nerve fibres with normal astrocytes (Fig. 3a, 3b, 3c, 3d). Normal pancreatic acini and pancreatic islet cells with intercalated and lobular ducts was observed in all the groups (Fig. 4a, 4b, 4c, 4d). All the groups showed normal cardiac muscle bundles with myocytes and no signs of damage or changes were observed (Fig. 5a, 5b, 5c, 5d). Congestion of alveolar tissue with normal alveolus was observed with low doses whereas mild infiltrations was observed in alveolar tissue with normal alveolar epithelium, alveolus and air spaces in high doses (Fig. 6a, 6b, 6c, 6d). All the treated groups
showed normal spleen with lymphoid aggregation (Fig. 7a, 7b, 7c, 7d). Normal ovarian stroma with corpus luteum and follicles were seen in all the treatment (Fig. 8a, 8b, 8c, 8d). Extract treated groups showed gastric glands with normal parietal cells and gastric mucosa (Fig. 9a, 9b, 9c, 9d). Normal columnar epithelial cells, intestinal villi, goblet cells were observed in intestine (Fig. 10a, 10b, 10c, 10d). Bone showed normal osteoblasts with bone matrix in all the extract treated groups (Fig. 11a, 11b, 11c, 11d). Control group animals showed normal hepatocytes with central vein, whereas those treated with low dose showed normal hepatocytes with mild congestion of blood vessels and edema. Dilatation of sinusoids with normal hepatocytes were seen with the medium dose treated animals. High dose group animals showed eosinophilic cytoplasm with mild infiltrations of mononuclear cells/heterochromatic nuclei (Fig. 12a, 12b, 12c, 12d). The interpretations of all the treated groups were done in comparison with control group.

**DISCUSSION**

Herbal medicines are believed to have lower risk compared to synthetic drugs, however in recent days the concept is becoming outdated since it is evident that herbal medicines also have potential risks. Various studies have reported the toxicity of herbal compounds and it becomes mandatory to rule out any such possible toxicity profile.32

The preliminary phytochemical analysis revealed the presence of many active constituents which may be pharmacologically beneficial. Animals were found to be free of any major toxic signs during as well as at the end of acute and sub chronic toxicity study. There were no abnormal signs of any motor and sensory functions.

Reduction of body weight is known as one of the most common index to understand toxicity profile of drugs.33 Minor insignificant changes in body weight were observed throughout the treatment period. Any changes in food intake or water drinking pattern indicates
toxic impact or abnormality on metabolism.\(^{32}\) Statistically insignificant changes in food and water intake indicate that the extract was safe on long term administration and did not induce any alteration in metabolic system.

The normal range of electrolytes, creatinine and urea indicates that ESW has no deleterious effect on renal system. Generally increased level of AST and ALT is considered to be an index of liver damage. Changes can occur in the level of these enzymes when there is changes in hepatic cellular permeability, damage to the hepatocytes or necrosis.\(^{34}\) Statistically insignificant results on ALT and AST ruled out any such toxic effects to liver. Reduction of glucose level seen in the present study is correlated to already reported alpha glucosidase as well as alpha amylase inhibitory activity of \(S.\ wightii\) and fucoidan.\(^{35-37}\) Therefore it is concluded that the hypoglycemic potential of extract of \(S.\ wightii\) is comparable to the studies previously reported on \(S.\ wightii\) or fucoidan.\(^{38,39}\)

Cholesterol is known to be one of the major lipids which can provide information about lipid metabolism.\(^{40}\) Few studies have already reported hypolipidemic property of sulphated polysaccharides, which could be correlated to the reduction in cholesterol level observed from the present study. Another study correlated the hypolipidemic effect of fucoidan to reduction in HMG CoA reductase expression and an upregulation of LDL receptor.\(^{41}\) Thus these results are complying with the previous studies available on hypoglycemic property of sulphated polysaccharides, which are expected to be the major compound present in \(S.\ wightii\) extract.\(^{42}\)

Hematological system is one of the most important system which serves as an indicator of the health status. It is also known to be an easy target for most of the toxic compounds.\(^{43}\) The results indicate that the extract of \(S.\ wightii\) is non-toxic to hematopoietic system.

Organ weight is known to be one of the main indices to derive any targeted organ toxicity. The absence of any significant differences in organ weight eliminates any such organ
level toxicity. No signs of abnormality or organ level damages were observed in macroscopic examination of gross anatomy. In histopathological examination, all the organs showed normal architecture. Though, some changes were observed, they were minimal and comparable with the observations of control group.

Thus, the present study reveals that ESW is safe up to 2000 mg/kg administered as single oral dose and long term administration of ESW does not cause toxicity to vital organs at submaximal doses, which encourages the long term use of ESW for any further pharmacological investigations.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Authors are thankful to Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences for providing required facilities and support.

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43. Lynch N, Berry D. Differences in perceived risks and benefit of herbal over the counter conventional and prescribed conventional, medicines and implication of this for safe and effective use of herbal products. Complement Ther Med. 2007;15:84-91.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Control) (g)</th>
<th>Group II (100 mg/kg) (g)</th>
<th>Group III (200 mg/kg) (g)</th>
<th>Group IV (400 mg/kg) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0th day</td>
<td>199.67±17.47</td>
<td>202.33±4.37</td>
<td>195.33±4.80</td>
<td>235.33±8.40</td>
</tr>
<tr>
<td>7th day</td>
<td>218.16±15.27</td>
<td>214±4.03</td>
<td>214±1.79</td>
<td>238.33±9.98</td>
</tr>
<tr>
<td>14th day</td>
<td>216.33±14.61</td>
<td>217.66±7.42</td>
<td>210.83±5.52</td>
<td>232.83±10.60</td>
</tr>
<tr>
<td>21st day</td>
<td>219.33±14.61</td>
<td>218.83±6.18</td>
<td>211.66±9.90</td>
<td>232±13.53</td>
</tr>
</tbody>
</table>

Table 1: Body weight changes with ESW treated rats
<table>
<thead>
<tr>
<th>28th day</th>
<th>224.66±5.65</th>
<th>224.67±5.65</th>
<th>215.33±16.95</th>
<th>234.5±15.56</th>
</tr>
</thead>
</table>

Values are expressed as Mean ± Standard Error of the Mean (SEM); (n=6).

### Table 2: Food intake in ESW treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Control) (g)</th>
<th>Group II (100 mg/kg) (g)</th>
<th>Group III (200 mg/kg) (g)</th>
<th>Group IV (400 mg/kg) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>22.68±0.64</td>
<td>24.26±0.49</td>
<td>23.41±0.97</td>
<td>23.1±1.08</td>
</tr>
<tr>
<td>Week 2</td>
<td>22.61±0.77</td>
<td>23.17±0.99</td>
<td>21.1±1.20</td>
<td>23.17±1.20</td>
</tr>
<tr>
<td>Week 3</td>
<td>21.58±1.89</td>
<td>20±1.12</td>
<td>19.95±1.59</td>
<td>19.05±1.57</td>
</tr>
<tr>
<td>Groups</td>
<td>Group I (Control) (ml)</td>
<td>Group II (100 mg/kg) (ml)</td>
<td>Group III (200 mg/kg) (ml)</td>
<td>Group IV (400 mg/kg) (ml)</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------</td>
<td>---------------------------</td>
<td>---------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Week 1</td>
<td>46.7±2.71</td>
<td>49.77±1.57</td>
<td>48.78±2.78</td>
<td>46.32±3.28</td>
</tr>
<tr>
<td>Week 2</td>
<td>50.02±2.40</td>
<td>52.66±1.93</td>
<td>51.21±4.2</td>
<td>53.69±2.77</td>
</tr>
<tr>
<td>Week 3</td>
<td>55.64±1.70</td>
<td>55.84±2.31</td>
<td>56.3±1.87</td>
<td>54.15±1.37</td>
</tr>
</tbody>
</table>

Values are expressed as Mean± Standard Error of the Mean (SEM); (n=6).

Table 3: Water Intake in ESW treated rats
Values are expressed as Mean± Standard Error of the Mean (SEM); (n=6).

Table 4: Biochemical parameters in ESW treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Control)</th>
<th>Group II (100 mg/kg)</th>
<th>Group III (200 mg/kg)</th>
<th>Group IV (400 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>82.98±2.80</td>
<td>46.17±2.56a</td>
<td>47.75±1.61a</td>
<td>36.49±1.74a</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>78±1.78</td>
<td>51.83±1.64b</td>
<td>52.5±1.92b</td>
<td>61.83±7.86</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.92±0.09</td>
<td>3.68±0.13</td>
<td>3.48±0.09</td>
<td>3.73±0.16</td>
</tr>
<tr>
<td>Parameter</td>
<td>Group I (Control)</td>
<td>Group II (100 mg/kg)</td>
<td>Group III (200 mg/kg)</td>
<td>Group IV (400 mg/kg)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>-----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.02±0.20</td>
<td>7±0.14</td>
<td>6.92±0.19</td>
<td>6.45±0.29</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.57±0.02</td>
<td>0.55±0.03</td>
<td>0.57±0.01</td>
<td>0.55±0.02</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>53.83±2.69</td>
<td>57.5±2.87</td>
<td>53.67±1.41</td>
<td>51.5±1.48</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>221.5±4.15</td>
<td>221±3.25</td>
<td>213.33±2.81</td>
<td>218.5±1.89</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>30.67±1.26</td>
<td>34.83±0.91</td>
<td>33±1.21</td>
<td>32.5±1.06</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>150.83±1.68</td>
<td>151.17±1.78</td>
<td>150.33±1.08</td>
<td>150.17±1.35</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.83±0.23</td>
<td>4.88±0.24</td>
<td>4.80±0.24</td>
<td>4.92±0.21</td>
</tr>
</tbody>
</table>

Values are expressed as Mean± Standard Error of the Mean (SEM); \( ^bP<0.01 \) (\(^cP<0.01 \) indicates significance with P value less than 0.01 when compared to control); \(^aP<0.001 \) (\(^dP<0.001 \) indicates high significance with P value less than 0.001 when compared to control); (n=6).

**Table 5: Hematological Parameters in ESW treated rats**


<table>
<thead>
<tr>
<th></th>
<th>Group-1 (control)</th>
<th>Group-2</th>
<th>Group-3</th>
<th>Group-4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBC (10^6/mm³)</strong></td>
<td>11.44±0.25</td>
<td>12.32±0.24</td>
<td>11.86±0.38</td>
<td>11.06±0.19</td>
</tr>
<tr>
<td><strong>WBC (10³/mm³)</strong></td>
<td>10.13±0.34</td>
<td>11.58±0.87</td>
<td>12.75±1.02</td>
<td>13.18±0.95</td>
</tr>
<tr>
<td><strong>Platelets (Lakhs/cu.mm)</strong></td>
<td>4.27±0.26</td>
<td>4.30±0.21</td>
<td>4.19±0.17</td>
<td>4.50±0.12</td>
</tr>
<tr>
<td><strong>Hb (g/dl)</strong></td>
<td>13.63±0.439</td>
<td>13.83±0.45</td>
<td>14.88±0.55</td>
<td>14.47±0.52</td>
</tr>
</tbody>
</table>

Values are expressed as mean± Standard Error of the Mean (SEM); (n=6).

Table 6: Urine analysis of ESW treated rats

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Animal groups</th>
<th>Urine Volume</th>
<th>pH</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group-1 (control)</td>
<td>1.5ml</td>
<td>7</td>
<td>Clear, pale yellow</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>Volume</td>
<td>Tissue Count</td>
<td>Color</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------</td>
<td>--------</td>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>2.</td>
<td>Group-2 (100 mg/kg)</td>
<td>2 ml</td>
<td>7</td>
<td>Clear, pale yellow</td>
</tr>
<tr>
<td>3.</td>
<td>Group-3 (200 mg/kg)</td>
<td>1.5 ml</td>
<td>7</td>
<td>Clear, pale yellow</td>
</tr>
<tr>
<td>4.</td>
<td>Group-4 (400 mg/kg)</td>
<td>1 ml</td>
<td>7</td>
<td>Clear, pale yellow</td>
</tr>
</tbody>
</table>

Table 7: Organ weights in ESW treated rats
<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (Control)</th>
<th>Group II (100 mg/kg)</th>
<th>Group III (200 mg/kg)</th>
<th>Group IV (400 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart (g)</td>
<td>0.833±0.042</td>
<td>0.792±0.044</td>
<td>0.733±0.038</td>
<td>0.742±0.022</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>0.71±0.029</td>
<td>0.841±0.019</td>
<td>0.818±0.042</td>
<td>0.873±0.064</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>8.41±0.572</td>
<td>7.40±0.425</td>
<td>7.81±0.701</td>
<td>8.60±0.972</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.853±0.058</td>
<td>0.855±0.103</td>
<td>0.812±0.058</td>
<td>0.795±0.064</td>
</tr>
<tr>
<td>Brain (g)</td>
<td>1.65±0.047</td>
<td>1.88±0.030</td>
<td>1.80±0.094</td>
<td>1.90±0.050*</td>
</tr>
<tr>
<td>Lungs (g)</td>
<td>1.69±0.096</td>
<td>1.95±0.153</td>
<td>2.16±0.305</td>
<td>2.04±0.277</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± Standard Error of the Mean (SEM). *P<0.05 (P<0.05 indicates significance with P value less than 0.05 when compared to control)
HISTOPATHOLOGY

1) TESTES

1a) Normal Control  
1b) SW 100 mg/Kg  
1c) SW 200 mg/Kg  
1d) SW 400 mg/Kg

2) KIDNEY

2a) Normal Control  
2b) SW 100 mg/Kg  
2c) SW 200 mg/Kg  
2d) SW 400 mg/Kg

3) BRAIN

3a) Normal Control  
3b) SW 100 mg/Kg  
3c) SW 200 mg/Kg  
3d) SW 400 mg/Kg

4) PANCREAS
5) HEART

5a) Normal Control
5b) SW 100 mg/Kg
5c) SW 200 mg/Kg
5d) SW 400 mg/Kg

6) LUNGS

6a) Normal Control
6b) SW 100 mg/Kg
6c) SW 200 mg/Kg
6d) SW 400 mg/Kg

7) SPLEEN

7a) Normal Control
7b) SW 100 mg/Kg
7c) SW 200 mg/Kg
7d) SW 400 mg/Kg

8) OVARY
8a) Normal Control  
8b) SW 100 mg/Kg  
8c) SW 200 mg/Kg  
8d) SW 400 mg/Kg

9) STOMACH

9a) Normal Control  
9b) SW 100 mg/Kg  
9c) SW 200 mg/Kg  
9d) SW 400 mg/Kg

10) INTESTINE

10a) Normal Control  
10b) SW 100 mg/Kg  
10c) SW 200 mg/Kg  
10d) SW 400 mg/Kg

11) BONE

11a) Normal Control  
11b) SW 100 mg/Kg  
11c) SW 200 mg/Kg  
11d) SW 400 mg/Kg

12) LIVER
**Groups**

Group I – Normal control vehicle treated 1% sodium CMC

Group II – Ethanol extract of *S. wightii* 100 mg/Kg (Low dose)

Group III – Ethanol extract of *S. wightii* 200 mg/Kg (Medium dose)

Group IV – Ethanol extract of *S. wightii* 400 mg/Kg (High dose)