Effects of Hibiscus Rosasinensis on the Hematology of the Swiss Albino Mice: An In-Vivo Study

Sonali Bhakta* and Shonkor Kumar Das

Abstract
The present research/study was conducted on the heamtology of the Swiss albino mice irrespective of sex and experiment was done by using the aqueous extract of Hibiscus rosasinensis. Nature has become a vital source of medicine nowadays and people are also becoming very much dependent on the herbal drugs. Hibiscus rosasinensis is a very common and renowned plant having a variety of medicinal uses. This plant parts (flower, leaf and shrub etc.) possesses many phytochemicals which have different medicinal values. But these chemical constituents whether affect the hematological parameters (Red Blood Cell, White Blood Cell, Hemoglobin Concentration and Packed Cell Volume) is yet to know. For the sake of this purpose the experiment was done. Hence, it is concluded that the use of Hibiscus rosasinensis whereas may not cause any adverse effect on the hematology of the rather it elevates the hematological parameters of Swiss albino mice.

Keywords: Hibiscus Rosasinensis, Hematology, Swiss Albino mice, In-vivo study

Introduction
For thousands of years, people have looked to natural means of healing. In developing countries of the world, most the people depend on herbal medical care [1]. The popularity of the traditional medicines is because of the belief that some diseases will only be cured by the traditional medicines.

Extracts of root, stem, bark, leaves and flowers of some medicinal plants have been shown to have activities against most dreaded pathogenic organisms like the bacteria, fungi etc, while some others are cytotoxic [2-10]. Besides, ingestion of some plant materials (either in the raw form or their extracts) having useful medicinal properties may cause anemia resulting from the sequestration of RBC in spleen, impaired red blood cell production or primary bone marrow dysfunction [11,12]. In the present study, an attempt is made to study the therapeutic value of the crude extract of two most common locally available ornamental plants flourishing throughout the year, namely Hibiscus rosasinensis. Keeping in mind the report of Williamsons, who has emphasized that the whole or partially purified extract of plant offer advantages over a single isolated ingredient [13]. In this investigation, we have sought to verify the effect of the administration of aqueous extract of H. rosasinensis flowers on few hematological parameters.

In search of new well-acceptable safe anti-fertility agent, the present study examined the effect of crude extract of the flowers of Hibiscus rosasinensis as an antifertility agent in oral route on male and female albino mice. Hibiscus rosasinensis Linn (Family: Malvaceae) is an ornamental evergreen plant with flowers widely distributed throughout Bangladesh [14]. All the parts of the plant have been used in traditional medicine [15]. Different literatures show that several indigenous plant products have antifertility activity; e.g. cotton seed oil extract, popularly known as gossypol and also piper longus, Olmium sandum and other ayurvedic ingredients. The oral administration of the aqueous extract of the flower of Hibiscus rosasinensis @ 500mg (1ml)/kg body weight is very much effective in case of male. But it does not decline the RBC, WBC, Hb (Hemoglobin) concentration and PCV rather maintains the upgrading rate [16].

Materials and Methods

Experimental animals
Twenty Swiss albino mice (Mus musculus) male and female were divided into control group (C) and treated group (T), which were purchased at the age of thirty days from the Animal Resource Center, International Centre for Diarrheal Disease Research, Bangladesh, Mohakhali, Dhaka. Before being used in the experiment, mice were reared for 15 days in order to be accustomed with the environment and also to reach the age of sexual maturity (since Swiss albino mice both male and female reach to their sexual maturity at 45-48 days). To observe the normal reproductive ability of the mice at the age of 45 days male and female mice were kept together and the first parity (first time delivery of off spring) was found at the age of 58 days (as the incubation period is 14 days). After that at the age of 60 days the experiment was started. The mice were housed in
compartmentalized rectangular metallic cages (9x11x7 cube inches) wrapped with wire mesh and also in the deep bottom dishes to facilitate the sexual behavior. The mice had neither developmental disorders, detectable genital diseases nor other diseases that may cause any problem in the experiment or affect the result thereby.

Rearing and care
The mice were cared at Animal Care Room, Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University in proper hygienic conditions, with experimental and normal feeding (standard pelleted feed for mice from ICDDR, B) ad libitum. During the experimental period, uniformity of the management practices was maintained. The ventilation of the rearing house of mice was sufficient as a standard one. The room temperature was 28±2°C and relative humidity 70-80% with natural day and light. Before starting the experiment the mice were reared and observed for a normal cycle to clarify that whether they were reproductive or not. For this reason both the male and female were kept together and fed the normal mice pellet and water ad libitum for at least 15 days.

Experimental Design
Twenty (20) mice were divided into two groups. The control (C) and the Treated (T), both the groups were given the same standard feed. But the mice of the treated group were given additionally extract of Hibiscus rosasinensis at a regular basis.

Control group (C): Normal feed and water
Treated group (T): Normal feed and water along with extract of Hibiscus rosasinensis

Experimental plant
Hibiscus rosasinensis is the experimental plant. It is a plant of Malvaceae family. The flower of the plant was used for the experiment. The flower consists of a variety of phytochemicals which are useful for many medicinal purposes.

Extraction of the flower
The plant material (flower) was collected and air dried for 10 days under a open shade and pulverized with the help of a mortar and pestle to fine powder. 500 mg of powder was dissolved in 30 ml of distilled water in a conical flask. The mixture was intermittently shaken throughout the period of extraction using glass rod stirrer, but allowed to stand overnight and filtered with what man filter paper No 1 into measuring cylinder and concentrated at 600°C in an incubator and next stored in a refrigerator at 4°C until used and modified by method described by Sagnuwan and Onyeyili.

Experimental treatment procedures
Before starting the experiment the mice were fed for 15 days by normal feed (pellet) and water ad libitum and the regular record of the feed consumption and body weight gain were recorded to be accustomed with the environment. This recording was started at the 45th day of age of the mice. At the age of 60th day the treated groups were administered the aqueous extract of the herbal products (flower of Hibiscus rosasinensis @ 4.4 mg/kg bwt.) orally for the purpose and on the previous day of treatment these groups were not given the 2nd meal. During the experimental period, the uniformity of the management practices was maintained. The experiment was continued for 1 month and after that the sample was collected from both the experimental and control the mice.

Sample collection
The sample was collected from both the control and treated groups. Blood sample was collected directly from the heart at Day 0 and Day 30 with the help of anticoagulant containing syringe to test the effect of flower extract of Hibiscus rosasinensis on WBC, RBC, Hb. conc. and PCV. The tests were done in the Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Results
The blood was collected from both the control and treated groups at day 0 and day 30 directly from the heart of the experimental mice. In the control group, all the hematological parameters (RBC, WBC, Hb. conc. and PCV) are usually in normal ranges.

In contrast to the treatment condition by the herbal extract at day 30, it was found that all the hematological parameters increased than the normal (as in day 0) in the treated group (T). The rise of the hematological parameters among the treated group increased comparing to the control group and it was less affected by the extract of flower of Hibiscus rosasinensis. This indicated that the herbal extract had the positive impact on the hematology and elevated or maintained the gradual up-gradation of the hematological parameters that is eventually beneficial in that particular period (Appendix III & IV).

Table 1: Hematological parameter (RBC) of the control at day 0 and treated at day 30

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Male</th>
<th>Percentage of increase</th>
<th>Female</th>
<th>Percentage of increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>4.87</td>
<td>58.93%</td>
<td>4.73</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Day 30</td>
<td>7.74</td>
<td></td>
<td>7.00</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2: Hematological parameter (WBC) of the control at day 0 and treated at day 30

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Male</th>
<th>Percentage of increase</th>
<th>Female</th>
<th>Percentage of increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>7.84</td>
<td>13.65%</td>
<td>7.09</td>
<td>10.7%</td>
<td></td>
</tr>
<tr>
<td>Day 30</td>
<td>8.91</td>
<td></td>
<td>7.85</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 3: Hematological parameter (Hb. Conc.) of the control at day 0 and treated at day 30

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Male</th>
<th>Percentage of increase</th>
<th>Female</th>
<th>Percentage of increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>6.00</td>
<td>50%</td>
<td>6.20</td>
<td>45.16%</td>
<td></td>
</tr>
<tr>
<td>Day 30</td>
<td>9.00</td>
<td></td>
<td>9.00</td>
<td></td>
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</tbody>
</table>

Table 4: Hematological parameter (PCV) of the control at day 0 and treated at day 30

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Male</th>
<th>Percentage of increase</th>
<th>Female</th>
<th>Percentage of increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>20</td>
<td>25%</td>
<td>21</td>
<td>23.81%</td>
<td></td>
</tr>
<tr>
<td>Day 30</td>
<td>25</td>
<td></td>
<td>26</td>
<td></td>
<td></td>
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</tbody>
</table>
Treatment of mice with crude extract of *Hibiscus rosasinensis* flowers (500 mg/kg BW) and Bougainvillea spectabilis leaves (800 mg/kg BW) for a period of 30 days indicates a significant increase in the level of hemoglobin and count of RBC. On the other hand, in B. spectabilis treated animals, the level of hemoglobin, RBC count & PCV declined significantly [17]. The study definitely points towards the potential role of the plant as anti-fertility agent [18].

The hematological parameters like MCH, MCHC and MCV are related to individual RBC, while Hb and PCV are associated with total population of RBCs. Therefore, if MCH, MCHC and MCV are not affected by the treatment with extracts, it means that neither the incorporation of Hb into RBC, nor the morphology and osmotic fragile of RBCs is altered [19,20].

Bhakta and Das, has already mentioned regarding the positive impact of the other herbal products like *Abrus Precatorius*, *Ricinus communis* and *Syzygium aromaticum* the positive impact on the hematology of the Swiss Albino mice [21]. Those mixtures of the herbal products also has been applied on the Swiss Albino mice both male and female and the same group has found that it has positive contraceptive impact on the testes and ovary of both male and female. They have also out Hibiscus as a purpose of the contraception and they have found the positive impact on the testes of the male mice as well as female ovary. At the same time this group has mentioned regarding the no baleful effect of this herbal product on the liver and kidney of the treated mice both the male and female [22-24]. So in total it could be said that this herbal product is safe to be used as a contraceptive agent.

Comparing with the other available commercial contraceptive agents (tablets or pills) this herbal product is far better to be used without any harmful effect on the body.

*Hibiscus rosasinensis* is a very potential herbal plant which has many medicinal uses. Though the effects of the plant on the hematology of the Swiss albino mice has been identified in this research that it does not affect the hematological parameters such as RBC, WBC, Hb.conc and PCV.

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**Author Contributions**
Sonali Bhakta carried out the experiments, analyzed the data and wrote the initial draft of the manuscript. Shonkor Kumar Das designed and supervised the research work and revised the manuscript, and finalized the manuscript. The manuscript was carefully read by both the authors before the submission process.

**Conflicts of Interest**
The authors declare that there is no conflict of interest towards the publication of this article.

**References**


