A new polyherbal contraceptive rendering positive effects on hematology in Swiss albino mice

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Abstract— Contraception is a way to prevent pregnancy. Hematology includes the study of etiology, diagnosis, treatment, prognosis, and prevention of blood diseases that affect the production of blood and its components, such as blood cells, hemoglobin, blood proteins, and the mechanism of coagulation. To find out the effect of herbal products on the blood parameters or hematology an experiment was conducted in the Bioresearch Laboratory, Dept. of anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh. The Swiss albino mice (both male and female) was the experimental animal, which were of 45-60 days of age and were brought from ICDDR,B, Mohakhali, Dhaka. They were divided into five groups among which one (1) was the control group and the rest four (4) were the treated groups as group A (Single dose Female), group B (Double dose Female), group C (Single dose Male) and group D (Single dose Male and Female both). The treatment was done by the mixture of three herbal extract (aqueous extract of the seed of Ricinus communis, Abrus precatorious and fruit of Syzygium aromaticum). The mixture was made by mixing 0.050 gm of each individual ingredient together making 0.150 gm/mice in 1ml of distilled water and fed to individual mice by dropper. The treatment was continued for 42 days and after that the mice were sacrificed ethically and the blood was collected for the hematological analysis. The hematological study that were done here: RBC, WBC, Hemoglobin concentration, and PCV. The RBC level was highest among the treated group D, the value was in male 8.66 and female 8.81. The WBC was highest among the group D and the value was 8.91 in male and 9.12 in female. The Hemoglobin concentration among the group was highest where the value was 9.0 in male and 9.12 in female. Among the group D the PCV value was highest that was 27% among both the male and female. The blood parameters among the treated groups increased comparing to the control group as well as the combination effect of the herbal products affect less than that of the single effect of the herbal products. Thus it can be predicted that the herbal does not hamper the proper increase of the blood parameter rather it increases or continues the upgrade of the blood parameters.

Index Terms— Contraception, hematology, polyherbal extracts, RBC, WBC, Hemoglobin, PCV and Swiss albino mice.

1 INTRODUCTION

It is eternal that nature makes our lives possible at every step as it is the chief depot of all the primary resources for human medicines. Herbal medicines can keep a person sound and healthy (1). Herbs are the major part of the human life. Moreover, people are becoming highly dependent on the herbal medicines rather than the synthetic or chemical drugs as herbs are usually free from any baleful effects. Herbal drugs have been used as medicines for treatment of a range of diseases since ancient times.

There are various methods of contraception having various types mode of action. Contraception generally prevents sperm from reaching and fertilizing an egg which is how a pregnancy starts. In the developing countries, most of the people depend on herbal medical care. Medicinal plants have a vital role to take care of the human life and making it healthy (2).

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A great number of plant species contain various chemical substances exhibiting health benefit properties, antioxidative, anti-inflammatory and antimicrobial effects. Their preventive and therapeutic use in animals is increasing day by day (3). Inspite of great advances observed in modern medicine in recent decades, plants still make an important contribution to healthcare; several dietary and herbal supplements can interfere with the efficacy of birth control pills. There are also many herbs that are very innocuous, which can be used by virtually anyone without any negative effects (4). Ricin and abrin are two members of class II ribosome inactivating proteins derived from castor beans (*Ricinus communis*) and rosary peas (*Abrus precatorius* (5).

The plant *Abrus precatorious* is popularly known as Rosary pea belong to the family leguminosae (Fabaceae), is found throughout India in hedges and bushes in exposed areas. Usually seeds are used against leucoderma, wounds, alopecia, asthma, tubercular glands, leprosy, fever, ulcer and tumor (6). Oil and crystalline steroidal fraction from the seeds of the Abrus has been reported as to possess significant antifertility activity(7).

2 METHODOLOGY

2.1. Experimental Animal

Swiss albino mice model was used for this experiment which were brought from ICDDR,B, Dhaka, Bangladesh. The onset of the experiment was 56 days as both the male and female mice reach to sexual maturity or puberty at this age. The average weight of each mouse was 34.02 gm (On an average). Total 30 mice were used in the experiment among them 12 were male and 18 were female.

2.2 Rearing and care of the experimental animal

The experimental (both the control and treated groups) mice were reared in the Lab animal house, Dept. of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, and supplied normal mice pellet and water ad-libitum. The room was well ventilated and temperature was maintained at 25-30^0 C (8). The uniformity of all the management practices was also maintained.

2.3 Experimental plants

The plants those were used for the purpose are: *Abrus precatorious* (Abrus), *Ricinus communis* (Ricin or Castor bean), *Syzygium cumenifer* (Clove) and *Hibiscus rosasinensis* (Joba). The seeds of the *Abrus precatorius* & *Ricinus communis*, fruit of *Syzygium cumenifer* and flower of *Hibiscus rosasinensis* were used in the extraction and to observe their effects in the respective organs.

2.4Extraction of the plants

The plant material (seed, fruit and flower) was collected and air dried for 10 days under an open shade and pulverized with the help of a mortar and pestle to fine powder. Fifty (50) gm of powder was dissolved in 1000 ml (1 L) of distilled water in a conical flask. The mixture was intermittently shaken throughout the period of extraction using glass rod stirrer and rotar evaporator but allowed to stand overnight and filtered with whatman filter paper No 1 into measuring cylinder and concentrated at 60^{0}C in an incubator and next stored in a refrigerator at 4°C until required for use (9) and modified by method described in (10).

2.5 Preparation of dose formulation

The mixture of three herbal products (Aqueous extract of the seed of *Ricinus communis, Abrus precatorious and* fruit of *Syzygium aromaticum*) and the last group was treated by the flower of *Hibiscus rosasinensis.* The mixture was made by mixing 0.050gm (11) of each individual ingredient together making 0.150gm/mice in 1ml of distilled water and then making an aqueous extract of it and fed to individual mice by dropper. The *Hibiscus* was also fed in the same way but the dose was 500mg/mice which were mixed in 1ml of distilled water.

First of all the seeds were meshed in to powder by mixture grinder after that the moisture from the seeds was removed by heating in the oven at high temperature. Then the powder was measured individually and then the three ingredients were mixed together in the above mentioned amount of water and mixed by the rotating evaporator. The aqueous extract of the *Hibiscus rosasinensis* was made in the same way maintaining the above mentioned dose.

2.6 Experimental design

The total mice both male and female were divided into 6 groups 1 control and other 5 treated. Each group having 3 females and 2 males. The groups were named like:

Control Group -They were fed normal feed and water adlibitum

Group A (Single dose female) -Treated by the herbal mixture on 0 day

Group B (Double dose female) -Treated by the herbal mixture on 0 and 7th day

Group C (Single dose male) - Treated by the herbal mixture on 0 day

Group D (Single dose male & female both) - Treated by the herbal mixture on 0 and 7th day

2.7 Experimental procedure

Before the experiment the mice were acclimatized to the laboratory condition for 10 days by providing standard diet and water ad libitum. During this period the regular record of the feed consumption and body weight gain was kept. This recording was started at the 50 days old of the mice. At the 60th day age of the mice of treated groups the treatment with the aforesaid extracts was started for birth control. The combined extract was give @ 1ml/mice and the aqueous extract was made by mixing 150 mg powder of each plant material, total 450mg/kg b.wt. The experimental mice were fasted over night before the experiment. The female mice were treated in Gr. A, Gr. B and male in Gr .C and at last in Gr. D and Gr. E both male and female were treated. On the 3rd day of first dose the male mice were given in the Gr. A and female in Gr. C and D as these male and female were kept separated during treatment. After 7 days of first dose the female mice of the Gr. B. During this period the normal feeding and body weight gain measurement were continued.

2.8 Collection of the sample

Blood samples were collected by cardiac puncture from mice (anaesthetized with diethyl ether) in anti coagulant (EDTA) containing test tubes for hematological parameter analysis (12, 13).

2.9 Determination of hematological parameters

Blood was collected in EDTA-containing sample bottles from the experimental animals for hematological analysis as recom-

mended by Malomo *et al.,* 2002. RBC and WBC counting was done with the help of changes in the Neubaur's chamber. Packed cell Volume (PCV) with Wintrobe hematocrit tubes and Haemoglobin (Hb) concentration by Sahli's method (14).

Fig. 1 Aneasthesia of the mice with ether in dessicator
Fig. 2 Blood collection
Fig. 3 Rearing of the animals
Fig. 4 After collection of the sample

3 RESULTS

Table 1 and 2 shows the haematological parameters in the experimental mice of the control group and treated groups before and after treatment. The changes are clearly visible here in these two tables, those are due to the treatment by different doses of the aforesaid 2 extracts of the 4 (four) plant materials (1 extract of the seed and fruit of the three plant materials and another extract by the flower of rest one) are presented in Table 1.

The Red Blood Cell (RBC) level in the control group was 4.87±0.10 million/ Cu mm in male and 4.73±0.03 million/Cu mm in female that is normal according to Pokharkar et al., (15), but among the treated groups, it increased comparing with the control group. At the day 42 (after treatment), the RBC value increased highly among the mice (both male and female) of Group D. In the male the value was 8.66±0.20 million/Cu mm and in the female the value was 8.81±0 million/Cu mm that is 77% increased among the treated groups than that of the Control (Fig 5).

The White Blood cell (WBC) level among the control group was 7.84±0 thousand/Cu mm in male and 7.09±0.0 thousand/Cu mm in female that is normal according to Pokharkar et al., (15) but in the treated groups it upgraded in comparison with the control group. In the treated groups the highest value was found in Group C (Male) and Group A (Female). The value for the male was 8.73±0.0 thousand/Cu mm and for the female was 9.12±0.12 thousand/Cu mm, which is approximately 28% higher than that of the control (Fig 5).

The hemoglobin concentration (Hb. Conc.) among the control group was normal that is 6.0±0.0 gm/dl in male and 6.2±0.2 gm/dl in female according to Pokharkar et al., (15) but the values changed among the treated groups after the treatment. It was increased in the treated groups and the highest value was found in the Group E that was treated by the *Hibiscus rosasinensis* and the value was 9.0 gm/dl in male and 9.2 gm/dl in female that is approximately 48 % than the control group. Fig 7 shows this feature. The packed cell volume (PCV) rate among the treated groups was found in an increased level than that of the control group as the value was normal in this group that is 20% in male and 21% in female.

The highest increased value of PCV among the treated groups was found in the male and female of Group D which was treated by the herbal extract. Both in male and female the value was 27%. This feature is shown in Fig 8. In the comparative study among the single dose of the herbal products on the hematology with that of the combined effect by the mixture asper the experiment is quite variable. The effects of Ricin, Abrin, Sygygium and Hibiscus in different treated groups were found as a combined product (Mixture) comparing to that of the individual. All the values of the hematologic parameter increased among the treated groups of the experiment than that of the literature but the exception was found only in the PCV value, which decreased in all the treated groups except the PCV value comparing to Ricin as per literature. Fig 8 expresses this feature.

Table 1.
Hematological parameters among the control and treated groups (at Day 0)

Parameters	Control Male	Control Female	Group A Female	Group B Female	Group C Male	Group D Male	Group D Female	Level of sig.
RBC	4.00±0.50b	4.20±0.20b	4.20±0.10b	4.30±0.20b	4.80±0.20a	5.00±0.30a	4.90±0.00a	**
WBC	5.50±0.50bc	5.20±0.20bc	5.30±0.30bc	6.10±0.10a	5.70±0.30ab	5.00±0.20c	5.10±0.10c	**
Hb Conc.	5.20±0.20d	5.50±0.50cd	6.10±0.10ab	6.20±0.20a	5.70±0.20bc	5.90±0.10bc	5.10±0.20d	**
PCV	22.00±2.00	23.00±3.00	21.00±1.00	21.00±2.00	22.00±2.00	22.00±1.00	24.00±2.00	NS

** = Significant at 5% level of probability, ** = Significant at 1% level of probability & NS = Not significant*

Table 2.

Hematological study among the control and treated groups (at Day 42)

Parameters	Control Male	Control Female	Group A Female	Group B Female	Group C Male	Group D Male	Group D Female	Level of sig.
RBC	4.87±0.10c	4.73±0.03c	5.61±0.10b	5.47±0.03b	5.70±0.30b	8.66±0.20a	**8.81±0.20a**	**
WBC	7.84±0.04d	7.09±0.09e	**9.12±0.12a**	8.40±0.40c	8.73±0.03b	7.96±0.04d	7.37±0.02e	**
Hb Conc.	6.00±0.20c	6.20±0.20c	7.40±0.40b	7.40±0.20b	7.40±0.10b	8.20±0.20a	**8.60±0.20a**	**
PCV	20.00±2.00b	21.00±2.00b	21.00±1.00b	22.00±2.00b	23.00±3.00ab	**27.00±3.00a**	**27.00±2.00a**	**

** = Significant at 5% level of probability, ** = Significant at 1% level of probability & NS = Not significan*

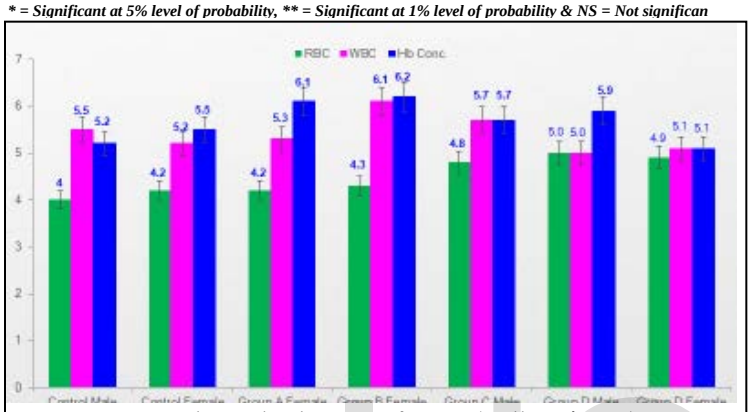

Fig. 5 Hematological changes {RBC (million/mm³), WBC (thousand/mm³), & Hb. conc. (gm%)} at Day 0

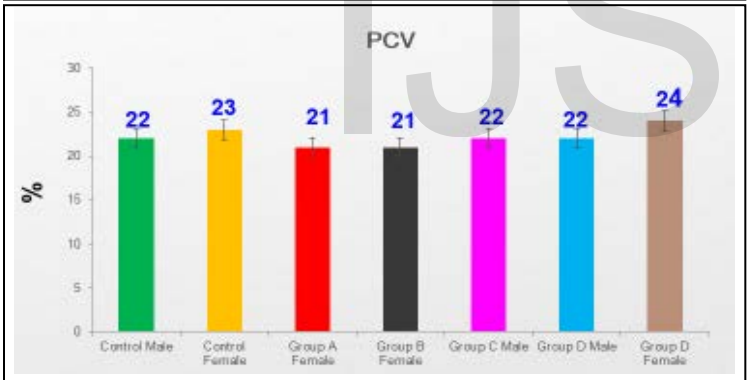

Fig. 6 Hematological changes (PCV %) at Day 0

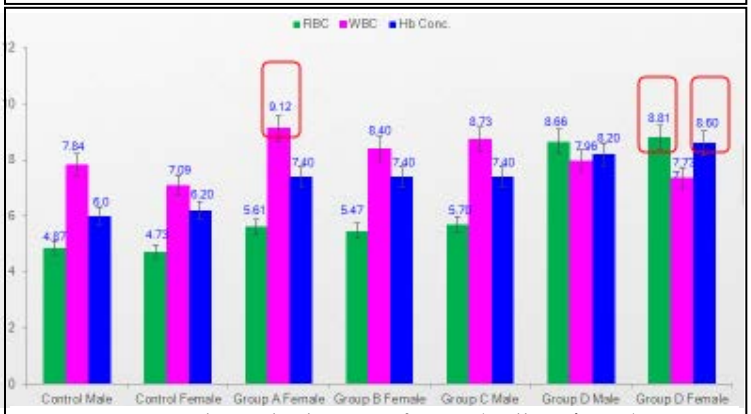

Fig. 7 Hematological changes {RBC (million/mm³), WBC (thousand/mm³), & Hb. conc. (gm%)} at Day 42

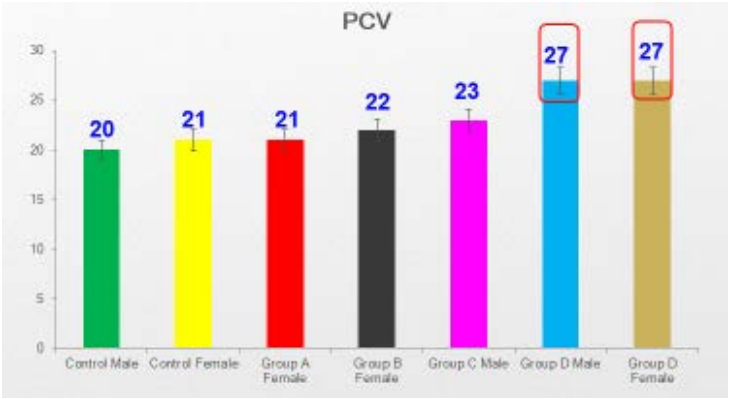

Fig. 8 Hematological changes (PCV %) at Day 42

4 DISCUSSIONS

The administration of any chemical compound for a long period might bring significant changes in the structure, function, metabolic transformation and concentration of biomedical enzymes and, even the metabolic pathways. These alterations may be rapid or slow and may lead to different biochemical mechanisms, producing a pathological state (16). But the treatment done by the herbal extract (aqueous extract of the seeds of *Ricinus communis, Abrus precatorious and* fruit of *Syzygium aromaticum*) and the aqueous extract of *Hibiscus rosasinensis* flower did not lower the blood parameter among the treated groups rather maintained the gradual increase the levels of the blood parameter such as RBC (Red Blood Cell), WBC (White Blood Cell), Hb. Conc. (Hemoglobin concentration) and PCV (Packed Cell Volume) which is eventually necessary in this particular time that is the pregnancy.

The RBC value was maintained in the control group as normal but it was in an increased level among the treated groups. Whether according to Pokkharkar (15) and Shama et al., (17) the hematological parameters must decrease than the normal or control among the treated groups. But the situation was quite reverse in this experiment. The gradual increased level of the RBC was much more among the male and female of Group D (Double dose male female both). While the value of the RBC was more among the male of the same group than that of the female wheather the same thing was also maintained among the other treated groups as well. So the RBC value increased much more among the treated groups those were treated by the herbal mixture.

The WBC value was also maintained as the normal among the control group that was not treated by the herbal mixtures rather given normal feed and water as per the need, but the value was in an gradual increased level among the treated groups. The value of the WBC increased much more among the male of the Group D whowere treated by the extract and also among the female of the Group B (Double dose female) who were treated by the herbal mixture.The rate was more among the female than that of the male (Female-9.12 thousand/Cu mm and among the Male-8.91 thousand/Cu mm).

The Hemoglobin concentration among the treated groups were in a

gradual increased level wheather among the control group it was in a normal level. The highest Hb Conc value was found among the male of Group D who were treated by the herbal mixtureand in female among the same Group D (In this group oth male and female were treated by the double dose of the herbal mixture). The Hb conc. among the male were more than that of the female (The value for male is 8.6 gm/dl wheather the value for the female is 8.2 gm/dl). The packed cell volume of the treated groups gradually increased wheather the value was normal in the control group. The value was same among the male and female of the treated groups. wheather the highest value was found among the Group D those were treated by the herbal extract (both the male and female were treated by the double dose of the aqueous extract of combined herbal products). The value of the Packed Cell Volume was 27% among both the male and female of group D. As a result we can say that these herbal products (seed, fruit and flower) do not have any negative impact on the hemic system of the body so that it cannot decrease the normal parameter of the hematology rather all these products will maintain the normal value and can increase the level as well. Prior to this research many other scientists or researchers have made various experiments with the above used herbal products. They observed the effects of these herbal products in a single pattern on mice and rats, but the effects of these seeds, fruit and flower on the hemic system of the body of Swiss albino mice was observed by me as a combined drug and the effects was quite positive comparing to that of the other chemical products as well as single action.

The administration of the aqueous extract of the seed of *Ricinus communis, Abrus precatorius and* fruit of *Syzygium aromaticum* does not act upon the normal hematology of the mice both male and female whenever the dose single or double. Even we know that during the pregnancy the Hb conc. and RBC rate decreases but here in our study we have found that the herbal products have maintained the gradual increase of the blood parameters which is the basic findings of my study. So these contraceptives restore the gradual elevation of the hematological parameters.

5 CONCLUSION

As there are various methods for contraception, present research was conducted to explore a new herbal contraceptive having positive effect on hematology. In this regard, aqueous extract of the seed of *Ricinus communis, Abrus precatorious* and fruit of *Syzygium aromaticum* was used. The blood parameters among the treated groups increased comparing to the control group and, the rise of hematological parameters are less affected by the combined extract treatment group than that of the single one. This indicates our new herbal product has positive effects on hematology and does not hamper the proper increase of the blood parameter rather it increases or continues the elevation of the blood parameters that is eventually necessary in that particular time.

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