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HAEMATOLOGICAL STUDIES IN RESPONSE TO LONG TERM GENISTEIN ADMINISTRATION IN ALBINO MICE, *Mus musculus.*

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ABSTRACT

Soy isoflavone genistein is a phytoestrogen that mediates its functions via interaction with estrogen receptor β like endogenous estradiol 17-β. This research was conducted to determine the effects of prolonged oral genistein administration on mice, with specific interest on haematological changes. Approximately 6-8 weeks old thirty mature male and female albino mice, *Mus musculus*, weighing approximately 30 ± 5 g were assigned to two groups, where the first served as the vehicle treated control (receiving 1:4 DMSO:PBS only) and second as the experimental group and received 10 mg genistein/kg body weight/day (dissolved in 1:4 DMSO: PBS) for 30, 60 and 90 days. Post treatment blood samples were collected and used for haematological studies like total count of Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb) content and differential count of WBC. Comparisons between control and treated animals (two way ANOVA analysis at $p<0.05$ to $p<0.001$) showed decreased RBC count, Hb content and neutrophil percentages, whereas WBC count, lymphocyte, eosinophil and monocyte percentages were increased after treatment. Together the results entail for no severe untoward effects on blood after prolonged daily genistein administration, unlike other exogenous estrogen analogues.

Key Words: Genistein, Mice, Haematology, Estrogen.

INTRODUCTION

Haematological study both in humans and in animal sciences is considered to be an important index of the physiological state of the individual. The primary goal of this study is to interpret the status of the blood profile during disease or clinical conditions in comparison to normal physiological conditions. It has already been proved that the blood picture may undergo huge changes during the life time (Khan and Zafar, 2005). The blood profile can undergo drastic changes with certain conditions such as, stress, infections and intoxications.

Genistein is a soy phytoestrogen and it is isoflavone in nature. Isoflavones are polyphenolic compounds found in many plant families, but especially in some members of the *Fabaceae* family. It is reported in several agriculturally important legumes such as soy, peanut, green peas, chick peas and alfalfa (Barnes, 2010 and Boué, 2003). Soy (*Glycine max*) beans are exceptionally rich in isoflavones, with an average content of 1-2 mg/gram (Coward *et al*., 1993) and constitute the major source for dietary isoflavones. Soy products are consumed in a variety of forms including whole beans, flour, soy protein isolates, textured soy protein, etc. (Barnes, 2010; Setchell, 2000). Fermented soy products like miso, tempeh and soy sauce are used regularly as part of East Asian cuisine (Barnes, 2010 and Coward *et al.*, 1993). Genistein is also termed as phytoestrogen as it shows estrogenic activities *in vivo*, and mediates its functions by interacting with estrogen receptor-β (Kim *et al.*, 1998).

Estrogen hormone is secreted by the ovary, testes, placenta and adrenal cortex. It has been shown to affect the skeletal system. Estrogen increases calcium deposition,

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accelerate epiphysis closure and increase bone formation. Estrogen has a slight anabolic effect and can increase sodium and water retention. Estrogens can also control release of gonadotropins from the pituitary gland which may cause inhibition of ovulation and inhibition of androgen secretion (Greenspan and Gardner, 2003). It has been reported by Perry and colleagues (2000), that high-dose of estrogen stimulate both new medullary bone formation in mouse long-bones and suppress hematopoiesis.

There had been numerous studies focusing on the estrogenic and /or anti-estrogenic properties of soy isoflavone genistein, but direct action on the blood profile have not been reported till date. Genistein being a phytoestrogen if is administered for longer durations it might act upon the estrogen receptors and in turn may affect the haematological parameters. Therefore the main aim of the study was to investigate the effect of prolonged exposure (up to 90 days) to phytoestrogen genistein (10mg/kg body weight/ day dissolved in 1:4 DMSO: PBS) on the haematological parameters.

MATERIALS AND METHODS

Animal model

Albino mice of both sexes approximately 7-8 week old, were housed and acclimated at approximately 24 ± 2 °C temperature with 10h : 14h light : dark cycle in the animal house of the Laboratory of Endocrinology, at the Department of Bioscience, Barkatullah University, Bhopal, Madhya Pradesh, India, and were provided commercially available mice feed and water *ad libitum*.

Chemical and dosage

Commercially available 98% pure HPLC grade Genistein powder purchased from Sigma Aldrich was used to prepare a dose containing 10 mg Genistein / kg body weight / day, dissolved in 1 : 4 Dimethyl sulfoxide (DMSO) : Phosphate buffered saline (PBS).

Fifteen males and females were administered orally 10 mg Genistein/kg body weight/day, dissolved in 1 : 4 Dimethyl sulfoxide (DMSO): Phosphate buffered saline (PBS) up to 30, 60 and 90 days (Experimental Group). Other fifteen of each sex received equal volume of vehicle (1: 4, DMSO : PBS) only for 30, 60 and 90 days served as the Control. Five mice from each group were sacrificed by cervical dislocation on day 31, 61 and 91 and the haematological studies were performed.

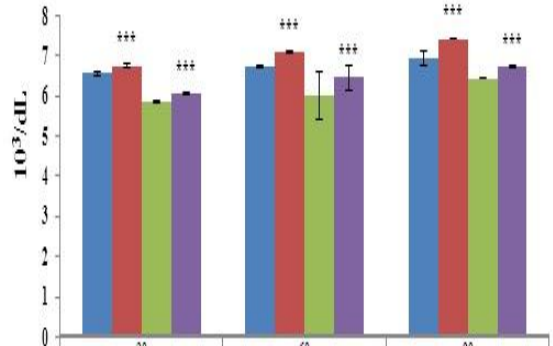
Haematology

Post sacrifice the blood was collected by cardiac puncture method in sterile blood collection vials containing anti-coagulant EDTA. Total count of Red blood cells (RBC), White blood cells (WBC) and differential leukocyte count (DLC) were determined according to the methods described by Coles (1980) and Haemoglobin (Hb) concentration (g/dL) was done according to Schalm *et al.*, (1975).

Statistical analysis (Zar, 2008)

All data obtained were expressed as mean and standard error of mean (Mean ± SEM). Differences within and between groups were tested for significance using analysis of variance (Two-way ANOVA- at $p < 0.05$ to $p < 0.001$).

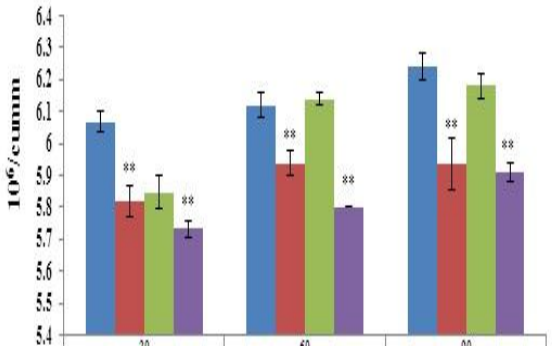
Figure 1: Comparison of Total WBC count (10^3/dL) between Genistein treated male and female *Mus musculus*.



	30	60	90
CONTROL MALE	6.556	6.744	6.93
EXPERIMENTAL MALE	6.748	7.1	7.43
CONTROL FEMALE	5.86	6.02	6.44
EXPERIMENTAL FEMALE	6.068	6.472	6.732

TREATMENT DURATIONS (IN DAYS)

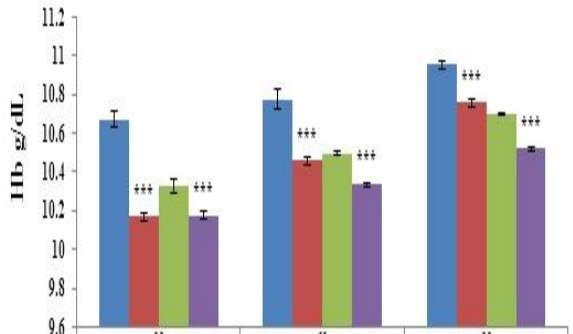
Figure 2: Comparison of Total RBC count (10^6/cumm) between Genistein treated male and female *Mus musculus*.



	30	60	90
CONTROL MALE	6.068	6.12	6.24
EXPERIMENTAL MALE	5.82	5.938	5.936
CONTROL FEMALE	5.848	6.14	6.18
EXPERIMENTAL FEMALE	5.732	5.8	5.91

TREATMENT DURATIONS (IN DAYS)

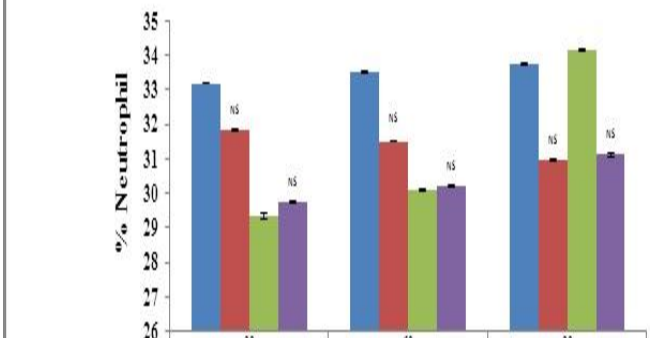
Figure 3: Comparison of Haemoglobin (Hb) concentration (g/dL) between Genistein treated male and female *Mus musculus.*



	30	60	90
CONTROL MALE	10.672	10.776	10.956
EXPERIMENTAL MALE	10.168	10.46	10.76
CONTROL FEMALE	10.33	10.496	10.696
EXPERIMENTAL FEMALE	10.176	10.334	10.52

TREATMENT DURATIONS (IN DAYS)

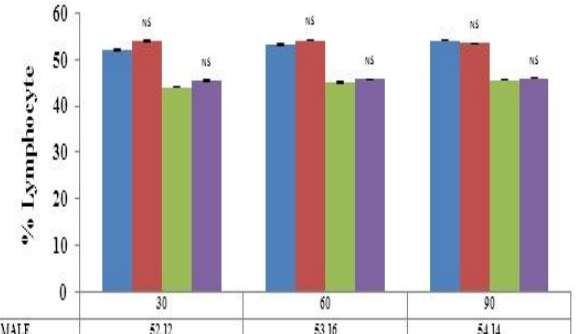
Figure 4: Comparison of Neutrophil (%) between Genistein treated male and female *Mus musculus.*



	30	60	90
CONTROL MALE	33.188	33.526	33.76
EXPERIMENTAL MALE	31.84	31.508	30.946
CONTROL FEMALE	29.326	30.09	34.14
EXPERIMENTAL FEMALE	29.726	30.2	31.12

TREATMENT DURATIONS (IN DAYS)

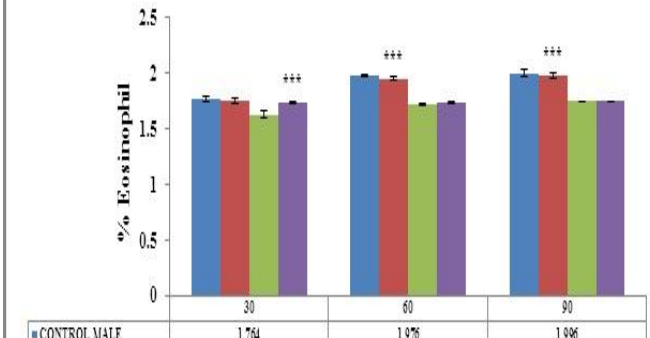
Figure 5: Comparison of Lymphocyte (%) between Genistein treated male and female *Mus musculus.*



	30	60	90
CONTROL MALE	52.12	53.16	54.14
EXPERIMENTAL MALE	54.09	54.14	53.5
CONTROL FEMALE	44.072	45.1	45.66
EXPERIMENTAL FEMALE	45.478	45.696	45.896

TREATMENT DURATIONS (IN DAYS)

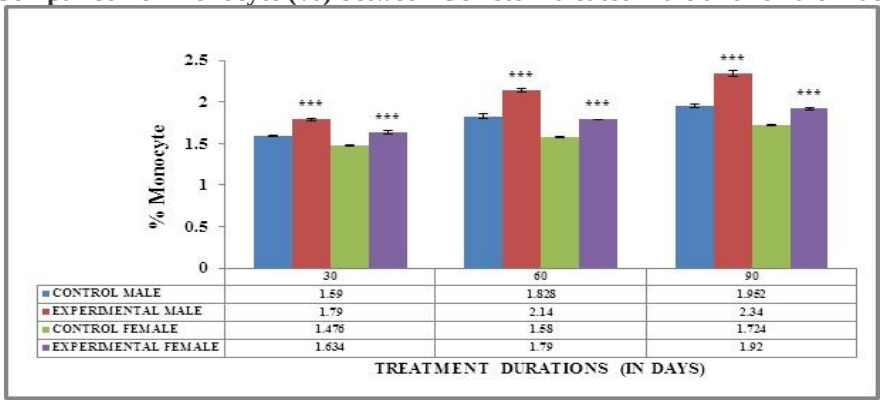
Figure 6: Comparison of Eosinophil (%) between Genistein treated male and female *Mus musculus.*



	30	60	90
CONTROL MALE	1.764	1.976	1.996
EXPERIMENTAL MALE	1.75	1.946	1.974
CONTROL FEMALE	1.624	1.718	1.742
EXPERIMENTAL FEMALE	1.726	1.73	1.744

TREATMENT DURATIONS (IN DAYS)

Figure 7: Comparison of Monocyte (%) between Genistein treated male and female *Mus musculus.*



	30	60	90
CONTROL MALE	1.59	1.828	1.952
EXPERIMENTAL MALE	1.79	2.14	2.34
CONTROL FEMALE	1.476	1.58	1.724
EXPERIMENTAL FEMALE	1.634	1.79	1.92

TREATMENT DURATIONS (IN DAYS)

*Values are expressed as Mean ± SEM; * = p<0.05; ** = p<0.01; *** = p<0.001; NS = Not significant.*

RESULTS

The effects of genistein administration on total WBC count of albino mice of both sexes show that there has been significant chronological increase in the values after treatment ($p<0.001$, two way ANOVA) in both males as well as females compared to the controls (Figure 1). There has also been significant chronological decrease in the total RBC count ($p<0.01$, two way ANOVA) post treatment in both males as well as females compared to the controls (Figure 2). In connection to the decrease in the total RBC count there also had been significant chronological decrease in the Hb concentration ($p<0.001$, two way ANOVA) after treatment with genistein in both males as well as females compared to the vehicle treated controls (Figure 3). The results also show that there was no significant changes ($p>0.05$, two way ANOVA) in the Neutrophil and Lymphocyte percentages (Figures 4 and 5). However there had been significant increase in the Monocyte percentage ($p<0.001$, two way ANOVA) in both males as well as females compared to the controls (Figure 7). The results show that there had been significant increase in Eosinophil percentage ($p<0.001$, two way ANOVA) between the control and experimental females of the 30 days group. However, the differences were significant between the vehicle treated control and treated males after 60 and 90 days of treatment ($p<0.001$, two way ANOVA). Although there was no significant differences recorded between the similar groups of females (Figure 6).

DISCUSSION AND CONCLUSION

Endogenous estrogen has been reported to increase lipid metabolism, sedimentation rate of RBCs and decrease total RBC count. Genistein has been reported to show estrogenic effects and therefore the decrease in erythrocyte count after genistein treatment can be accounted for the increase in plasma lipoproteins *i.e.*, hyperlipaemia due to genistein administration, similar to that caused by endogenous estrogen (Greenspan and Gardner, 2003). Estrogen thus may cause haemodilution by increasing the plasma volume, which may be one of the causes behind the fall in RBC count (Sandabe and Timothy, 2007). Gilbert (1962) and Nirmalan and Robinson (1972) reported decrease in total RBC count in broiler chicken, post exogenous estrogen treatment. Treatment with Stilbesterol in female albino rats have shown to decrease the total RBC counts, Haemoglobin concentration, WBC count and decrease in the percentage of neutrophils, lymphocytes, monocytes whereas there was increase in eosinophil percentage after 7-21 days (Sandabe and Timothy, 2007). Stilbesterol is also an estrogen analogue that works like estrogen in circulation. The depletions of RBC and Haemoglobin content is at par with the situations where estrogens cause hyperliaemia and haemodilition. According to Ugochukwu and colleagues (2008), estrogen may down regulate the expression of adhesion and chemokine molecules in response to inflammation in many animals. Therefore it may be one cause behind the increase in the number of WBC after prolonged genistein (a phytoestrogen) administration. Researchers have also reported that estrogen treatment alters the recruitment and adhesion of leukocytes to the endothelium, which was induced by inflammation promoters that offer a possible mechanism by which estrogen exert an anti-inflammatory effect. These effects of estrogens were due to aiming at the interaction of monocytes with the vascular endothelium (Nilsson, 2007).

Deficiency in gonadal steroids cause reduction in osteoblastic activity and increase in adipocytes in bone marrow, and therefore changes cell differentiation as a whole (Pedawy, 2009). The increase in WBC count after prolonged exposure to genistein (up to 90 days) at a dose of 10mg/kg body weight/ day can be acclaimed to be one of the basic defense mechanisms of body raised against any exogenous material. None the less, genistein administration for 90 days has shown marked increase in WBC count and monocyte percentage. Although there was marked increase in eosinophil percentage after 30 days treatment in females and 60 and 90 days treated males as compared to vehicle treated control. Genistein, an analogue to endogenous estrogen may therefore be claimed to alter the haematological parameters in a way that it may be considered non-harmful to mice. Therefore in fine it may be concluded that prolonged genistein administration causes sensitization of the defense system of body but in such a way that it does not alter the blood picture severely as compared to that of the normal animals.

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