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Research Article

EFFECT OF SOME HERBAL DRUGS ON HAEMATOLOGICAL PROFILES OF RATS

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ABSTRACT

Excessive use of oestrogen or ethinyl oestradiol (EO, a semisynthetic 17β-oestradiol as highly potent oestrogen) can cause many detrimental effects, including cancer in humans as well as in animals. On the other hand, many herbal drugs are beneficial for the treatment of toxicity and cancer. Likewise, ProImmu and its plant-ingredients have been reported to possess immunomodulatory effect and restore the normal histoarchitecture of the damaged tissues. In the present study, the beneficial effects of ProImmu, and its two ingredients, viz., *Tinospora cordifolia* and *Withania somnifera* have been observed against EO altered haematological profiles in the female albino rats. The haematological study showed that EO (@ 250 µg/kg, orally, thrice a week) altered levels of haemoglobin (Hb), total leucocyte count (TLC) and differential leucocyte count (DLC- lymphocyte, monocyte, neutrophil and eosinophil) could be significantly normalized by ProImmu (@ 150 mg/kg, orally, daily for 8 weeks); however, the normalization of these parameters brought up by *T. cordifolia* (@ 250 mg/kg, orally, daily for 8 weeks) and *W. somnifera* (@ 250 mg/kg, orally, daily for 8 weeks) was found to be of lesser degree. ProImmu (@150 mg/kg, orally, daily for 12 weeks), *T. cordifolia* (@250 mg/kg, orally, daily for 12 weeks) and *W. somnifera* (@ 250 mg/kg, orally, daily for 12 weeks) also caused the normalcy of these parameters, but to less extent than observed on the 8 weeks.

KEYWORDS: Ethinyl oestradiol (oestrogen), herbal drugs, haematological profiles, ProImmu, *Tinospora cordifolia, Withania somnifera.*

INTRODUCTION

Oestrogen has been stated to cause many detrimental effects both in humans and animals. The over doses of oestrogen may cause nausea, vomiting, anorexia, migraine, blurring of vision, mental depression, headache, asthma, endometriosis, fibroids, breast engorgement, increased vaginal secretion, oedema, cardiovascular and hepatic diseases, cancer, stroke, Alzheimer's disease, and many others in human beings[1-3]. Synthetic oestrogen, hexoestrol (60 mg/kg, orally, daily up to 40 days) is hepatotoxic in female rats[4]. All the rats showed reduction in body weight and appetite, and gain in liver weight. This latter effect was due to both cellular hypertrophy and hyperplasia. The adverse effects like bone marrow suppression, pyometra and infertility in bitches after treatment with many oestrogen preparations[5]. Oestrogen caused lethargy, anorexia and collapse with clinical findings, including vaginal discharge, endometritis and enlarged mammary glands in bitches[6]. Ethinyl oestradiol (EO, a semisynthetic 17β-oestradiol which is highly potent oestrogen) in doses of 250, 500 and 750 µg/kg, orally, weekly has been reported to cause uterine and ovarian cytotoxicity and cancer in albino rats[3].

Several ethnomedicinal plants and their preparations are being used for prevention and treatment of cytotoxicity caused by various toxicants. According to WHO estimates, more than 80% people depend on autochthonous herbal drugs for their primary health needs[7]. ProImmu (a herbal drug manufactured by Indian Herbs Research & Supply Co. Ltd., Saharanpur, UP), which contains *Tinospora cordifolia* (Giloe), *Withania somnifera* (Ashwagandha), *Ocimum sanctum* (Tulsi) and *Emblica officinalis* (Amla), has been reported to possess immunomodulatory effect and restore the normal histoarchitecture of the damaged tissues[3,8]. Cytogenic effect of ProImmu has been observed against EO induced damage in the uterine tissues of rat[9].

In view of above facts, the present study was undertaken to evaluate the beneficial effects of three herbal drugs, viz., *T. cordifolia, W. somnifera* and ProImmu against EO (oestrogen) induced haematological alteration in female albino rats.

MATERIALS AND METHODS

To evaluate the beneficial effects of herbal drugs *T. cordifolia, W. somnifera* and ProImmu, fifty four female albino rats were used in the study. Rats were divided into 9 groups, each consisted of six rats. The rats of group 1 were administered with normal saline to serve as normal group. EO (250 µg/kg, orally, thrice a week) was administered in groups 2 to 5 and groups 6 to 9 of rats for 8 and 12 weeks, respectively. However, the hydroalcoholic extract (HAE) of *T. cordifolia* (250 mg/kg, orally, daily), HAE of *W. somnifera* (250 mg/kg, orally, daily) and aqueous suspension of ProImmu powder (150 mg/kg, orally, daily) were administered to the rats of groups 3 to 5, respectively for 8 weeks. Similarly, these three herbal drugs at the same dose rate were administered to the rats of groups 7 to 9, respectively for 12 weeks.

After end of the experiment, the haematological study in the blood samples of all the rats was conducted. The blood was collected from the eye veins (Intraocular method) of rats. The haematological parameters, viz., Hb, TLC and DLC (viz., lymphocyte, monocyte, neutrophil, eosinophil and basophil) have been estimated according to the methods described by Jain[10]. For statistical analysis, the data of each parameter were analyzed and the significance of difference was

determined by employing the Duncan's new multiple range test at P = 0.05 (5% level of significance)[11].

Table 1: Effect of *T. cordifolia, W. somnifera* and ProImmu on Hb, TLC and lymphocyte in ethinyl oestradiol induced damage in rat

Group	Treatment	Week of experiment	Hb Mean** ±S.E. (g/dl)	TLC Mean** ±S.E. (10^3/µl)	Lymphocyte Mean** ±S.E. (%)
1	Normal saline	1st	13.7^{a}±1.4	7766.7^{a}±647.9	36.8^{a} ±1.0
2	EO @ 250 µg/kg, orally, thrice a week for 8 weeks	9th	10.0^{b}±0.3	3800.0^{d}±57.7	26.3^{ef}±0.9
3	EO according to group 2 and *T. cordifolia* @ 250 mg/kg, orally, daily for 8 weeks from the start of experiment	9th	12.5^{a}±0.2	5866.7^{bc}±566.1	31.5^{cd}±0.8
4	EO according to group 2 and *W. somnifera* @ 250 mg/kg, orally, daily for 8 weeks from the start of experiment	9th	12.6^{a}±0.2	6316.7^{abc}±6892	33.0^{bcd}±0.82
5	EO according to group 2 and ProImmu @ 150 mg/kg, orally, daily for 8 weeks from the start of experiment	9th	13.0^{a}±0.3	7300.0^{ab}±587.7	35.5^{ab}±1.1
6	EO @ 250 µg/kg, orally, thrice a week for 12 weeks	13th	7.8^{c}±0.3	3150.0^{d}±76.4	20.0^{f}±0.6
7	EO according to group 6 and *T. cordifolia* @ 250 mg/kg, orally, daily for 12 weeks from the start of experiment	13th	12.0^{a}±0.2	5300.0^{c}±258.2	29.5^{de}±1.2
8	EO according to group 6 and *W. somnifera* @ 250 mg/kg, orally, daily for 12 weeks from the start of experiment	13th	12.3^{a}±0.2	5716.7^{bc}±463.6	30.0^{de}±0.9
9	EO according to group 6 and ProImmu @ 150 mg/kg, orally, daily for 12 weeks from the start of experiment	13th	12.8^{a}±0.3	6483.3^{abc}±489.9	34.5^{abc}±0.8

*Number of animals in each group=6.

**Mean with same superscript does not differ significantly (Duncan's new multiple range test at P=0.05).

Table 2: Effect of *T. cordifolia, W. somnifera* and ProImmu on monocyte, neutrophil and eosinophil in ethinyl oestradiol induced damage in rat

Group*	Treatment	Week of experiment	Monocyte Mean** ±S.E. (%)	Neutrophil Mean** ±S.E. (%)	Eosinophil Mean** ±S.E. (%)
1	Normal saline	1st	7.0^{a}±0.4	63.3^{a}±2.5	4.1^{e}±0.5
2	EO @ 250 µg/kg, orally, thrice a week for 8 weeks	9th	1.5^{f}±0.2	37.0^{d}±0.5	6.5^{b}±0.4
3	EO according to group 2 and *T. cordifolia* @ 250 mg/kg, orally, daily for 8 weeks from the start of experiment	9th	3.5^{cde}±0.4	49.2^{c}±0.8	5.0^{cde}±0.6
4	EO according to group 2 and *W. somnifera* @ 250 mg/kg, orally, daily for 8 weeks from the start of experiment	9th	4.2^{bcd}±0.6	54.8^{b}±2.5	4.8^{cde}±0.3
5	EO according to group 2 and ProImmu @ 150 mg/kg, orally, daily for 8 weeks from the start of experiment	9th	5.5^{b}±0.4	61.0^{a}±1.2	4.3^{de}±0.3
6	EO @ 250 µg/kg, orally, thrice a week for 12 weeks	13th	1.3^{f}±0.2	31.2^{e}±1.1	8.0^{a}±0.4
7	EO according to group 6 and *T. cordifolia* @ 250 mg/kg, orally, daily for 12 weeks from the start of experiment	13th	2.3^{ef}±0.2	45.3^{c}±1.7	6.0^{bc}±0.4
8	EO according to group 6 and *W. somnifera* @ 250 mg/kg, orally, daily for 12 weeks from the start of experiment	13th	3.3^{de}± 0.5	47.0^{c}±1.7	5.5^{bcd}±0.2
9	EO according to group 6 and ProImmu @ 150 mg/kg, orally, daily for 12 weeks from the start of experiment	13th	4.7^{bc}±0.4	56.8^{b}±1.9	4.5^{de}±0.4

*Number of animals in each group=6.

**Mean with same superscript does not differ significantly (Duncan's new multiple range test at P=0.05).

RESULTS AND DISCUSSION

The Hb concentrations, TLC and lymphocyte counts observed in the rats of groups 1 to 9 are presented in Table 1. The Hb values of groups 1, 5, 9, 4, 3, 8 and 7 did not decrease significantly (P<0.05) and found non-significant with each other. However, the Hb values of these groups decreased significantly with groups 2 and 6. The Hb values of groups 2 and 6 also decreased significantly with each other. The per cent decrease in the Hb values was found to be 27.0, 8.8, 8.0, 5.1, 43.1, 12.4, 10.2 and 6.6 in groups 2 to 9, respectively as compared to group 1. There was found no significant difference amongst the TLC values of groups 1, 5, 9 and 4; groups 5, 9, 4, 3 and 8; groups 9, 4, 3, 8 and 7; groups 2 and 6. However, the TLC value differed significantly (P<0.05) in the remaining groups, viz., group 1 with group 3, 8, 7, 2 and 6; group 5 with groups 7, 2 and 6; groups 9, 4, 3, 8 and 7 with groups 2 and 6. The per cent decrease in the TLC values was found to be 51.1, 24.5, 18.7, 6.0, 59.4, 31.8, 26.4 and 16.0 in groups 2 to 9, respectively as compared to group 1. The lymphocyte values of groups 1, 5 and 9 did not decrease significantly (P<0.05) and found non-significant with each other. Similarly, the lymphocyte value of groups 5, 9 and 4 did not decrease significantly with each other; whilst such values of groups 9, 4 and 3; groups 4, 3, 8 and 7; groups 8, 7 and 2; groups 2 and 6 did not decrease significantly with each other. However, the lymphocyte

values of group 1 with groups 4, 3, 8, 7, 2 and 6; group 5 with groups 3, 8, 7, 2 and 6; group 9 with groups 8, 7, 2 and 6; groups 4 and 3 with groups 2 and 6; groups 8 and 7 with group 6 differed significantly ($P<0.05$). The per cent decrease in the lymphocyte values was found to be 28.5, 14.4, 10.3, 3.5, 45.7, 19.8, 18.5 and 6.3 in groups 2 to 9, respectively as compared to group 1.

The concentrations (%) of monocyte, neutrophil and eosinophil estimated in the rats of groups 1 to 9 are depicted in Table 2. The monocyte value of group 1 differed significantly ($P<0.05$) with those of groups 2 to 9. Amongst the latter groups, the monocyte values differed significantly as under: group 5 with groups 3**,** 8, 7, 2 and 6; group 9 with groups 8, 7, 2, 6; group 4 with groups 7, 2, and 6; groups 3 and 8 with groups 2 and 6; groups 2 and 6 with all other groups except group 7. However, there was no significant difference amongst the remaining groups. The per cent decrease in the monocyte values was found to be 78.6, 50.0, 40.0, 21.4, 81.4, 67.1, 52.9 and 32.6 in groups 2 to 9, respectively as compared to group 1. The neutrophil values of groups 1 and 5 decreased significantly ($P<0.05$) in all other groups. Similarly, such values of groups 9 and 4 differed significantly with groups 3, 8, 7, 2 and 6; while the neutrophil values of groups 3, 8 and 7 differed significantly with groups 2 and 6. There was also found a significant difference between the neutrophil values of groups 2 and 6. The neutrophil values of groups 1 and 5; groups 9 and 4; groups 3, 8 and 7 did not differ significantly with each other. The per cent decrease in the neutrophil values was found to be 41.5, 22.3, 13.4, 3.6, 50.7, 28.4, 24.6 and 10.3 in groups 2 to 9, respectively as compared to group 1. The eosinophil values of group 1 increased significantly ($P<0.05$) in groups 8, 7, 2 and 6. Such value of group 6 decreased significantly in all other groups. There was found no significant difference amongst the eosinophil values of groups 1, 5, 9, 4 and 3; groups 5, 9, 4, 3 and 8; groups 4, 3, 8 and 7; groups 8, 7 and 2. The per cent increase in the eosinophil values was found to be 58.5, 21.9, 17.0, 12.2, 95.1, 46.3, 34.1 and 9.8 in groups 2 to 9, respectively as compared to group 1. The basophil (%) in normal rats was found to be zero. Therefore, basophil in treated groups (2-9) was not counted.

The haematological study conducted in the present work showed that EO altered levels of many parameters could be significantly normalized by ProImmu (150 mg/kg, orally, daily for 8 weeks); however, the normalization of these parameters brought up by *T. cordifolia* (250 mg/kg, orally, daily for 8 weeks) and *W. somnifera* (250 mg/kg, orally, daily for 8 weeks) was found to be of lesser degree. Further, ProImmu (150 mg/kg, orally, daily for 12 weeks), *T. cordifolia* (250 mg/kg, orally, daily for 12 weeks) and *W. somnifera* (250 mg/kg, orally, daily for 12 weeks) also caused the normalcy of these parameters, but to less extent than observed on the 8 weeks. The findings of the present study are in accordance with the many investigators. The immunomodulatory effect of *T. cordifolia* was observed by earlier workers[12]. The methanolic extract (200 mg/kg, ip, daily for 5 days) of *T. cordifolia* stem increased the humoral immune response and reduced solid tumour growth in mice[13]. The whole plant extract of *W. somnifera* possesses antioxidant, antiinflammatory, immunomodulating and antistress properties[14]. It has also been reported that *W. somnifera* root is a potential source of hypoglycaemic, diuretic and hypocholesterolemic drugs[15]. Methanol and hexane extracts of leaves and roots of *W. somnifera* showed potent antibacterial activity as well[16]; however, the leaf, root and root bark of this herb has been shown to possess antimalarial activity[17.]

CONCLUSION

On the basis of haematological study, it can be concluded that ProImmu, *T. cordifolia* and *W. somnifera* possess the cytogenic effect, which is time dependent. The cytogenic effect of ProImmu may be due to its plant-ingredients, which have been reported as potent immunostimulatory and antioxidant plants. The antioxidants phytochemicals cure many diseases by protecting the cells from damage caused by 'free radicals'- the highly reactive oxygen compounds. The cytogenic effects and mechanism of action of ProImmu, *T. cordifolia* and *W. somnifera* may be postulated on the basis of their immunostimulatory, antioxidant, phagocytic and other cytoprotective activities.

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