INTRODUCTION

Currently available oral contraceptives are associated with several side effects such as hormonal imbalance, weight gain, hypertension, and increased risk of cancer [1], besides being expensive and not completely reliable, thus limiting their use. There is a growing need for alternative antifertility strategies which are safer, cost-effective, self-administrable, and effective. Plant-based antifertility drugs may be looked on as a potential antifertility agent for controlling global population, especially in the developing and underdeveloped nations [2].

Trillium govanianum is an important medicinal herb found at an altitude of 2400–3500 m in the Himalayan regions. Rhizomes of T. govanianum are a rich source of steroids, such as trillarin, which on hydrolysis yield diosgenin, a corticosteroid hormone used in preparation of steroidal medicines and sex hormones, such as testosterone, glucocorticoids, and progesterone besides being useful in rheumatism and regulation of menstrual flow [3,4]. T. govanianum is used in several traditional and folk medicines containing steroids and sex hormones and for the treatment of ailments such as boils, dysentery, inflammation, menstrual disorders, sex-related disorders, wound healing, and as an antiseptic [5-7]. The antioxidant, analgesic, anti-inflammatory, anticancer, and antifungal potential of T. govanianum has been experimentally validated [4,5,8-10]; however, the scientific evidence to justify its antifertility potential is lacking. Therefore, the present study was aimed to investigate the antifertility potential of T. govanianum and to elucidate the possible mechanism.
for implantation sites. Ovaries and uterus were fixed in 4% formalin for histopathological examination. Serum was extracted and used for estrogen evaluation [12].

**Estrogenic/antiestrogenic study**

Estrogenic/antiestrogenic effect of plant extract was evaluated in the ovarioctomized female Wistar rats, as per the method described by Taprial et al. [12]. Animals were divided into six groups (n=5). Group-1: Control (received vehicle); Group-2 received 17α-ethinylestradiol (EE; 1 μg/rat/day; S.C.); Group-3 and Group-4 received 125 and 250 mg/kg TGE, respectively (suspended in vehicle; orally); and Group-5 and Group-6 received 125 and 250 mg/kg TGE, respectively (suspended in vehicle; orally) along with EE (1 μg/rat/day). All the treatments were continued for 11 days. Animals were sacrificed on day 12, body weight changes were observed, serum was extracted, and estrogen levels were determined [12].

**Statistical analysis**

Results are depicted as mean±standard deviation. Statistical significance was determined using one-way ANOVA followed by Dunnnett’s multiple comparison test, using GraphPad prism 5 software. All the results were compared to control, and the statistical significance was determined at *p<0.05, **p<0.01, and ***p<0.001.

**RESULTS**

**Phytochemical screening**

Phytochemical screening of the TGE revealed the presence of alkaloids, glycosides, flavonoids, tannins, steroids, amino acids, lignin, and inulin in the extract. Volatile oil, aleurone grains, fats, and fixed oils were not present in the plant extract.

**Anti-implantation activity**

Results of anti-implantation effect of TGE are depicted in Table 1. Control animals demonstrated intact fertility and were observed to have 7.8±0.84 implantation sites. A number of implantation sites in TGE-treated animals were significantly (p<0.001) reduced in dose-dependent manner. We observed 82.05% and 100% inhibition of implantation at 125 and 250 mg/kg dose, respectively. Further, TGE treatment significantly elevates serum estrogen levels, 106.72 and 216 pg/ml at 125 mg/kg and 250 mg/kg extract treatment, respectively, when compared to control (100.84 pg/ml). TGE treatment resulted in a significant (p<0.001) increase in uterus weight when compared to control animals. These results suggest that TGE possesses strong anti-implantation effect and may elevate serum estrogen levels.

**HISTOPATHOLOGY RESULTS**

Results of the histopathological examination of the uterus and ovary of the experimental animals are depicted in Fig. 1. Our results demonstrated a dose-dependent thickening of uterine endometrium wall when compared to control, which might have contributed in the anti-implantation effect of TGE. The ovaries appeared healthy and showed morphological resemblance with control animals; however, a slight thickening in the ovarian walls was observed in animals treated with 250 mg/kg TGE.

**Estrogenic/antiestrogenic study**

Estrogen levels were found to be significantly (p<0.001) higher in animals treated with 1 μg EE when compared to control. Treating animals with TGE resulted in a significant (p<0.001) increase in serum estrogen level, which was observed to be 446.21 pg/ml and 436.2 pg/ml at 125 and 250 mg/kg dose, respectively (Table 1). We observed significantly (p<0.001) higher estrogen levels in animals treated with plant extract and EE; however, there was a significant difference between the extract treatment groups and EE group. These results suggest that TGE is having strong estrogenic effect, which appears to be saturable. Moreover EE, TGE, and TGE+EE treatments significantly (p<0.01) increased the body weight and uterus weight of animals (Table 1) when compared to control.

**DISCUSSION**

The traditional use of herbal remedies has high fascination but lack scientific justifications, and very few remedies have been explored for their efficacy and mechanism of action [13]. Rhizomes of T. govanianum are a rich source of steroids and have been used traditionally during menstrual disorders and in the treatment of sex-related disorders in several folk medicines [5-7]. In the present study, we evaluated the anti-implantation effect of TGE through anti-implantation studies and investigated the potential mechanism. TGE treatment demonstrated a strong dose-dependent anti-implantation effect that could be attributed to its potential to induce endometrial thickening and estrogenic effect. Previously, anti-implantation effects of plant extract have been attributed to endometrial thickening and changes in estrogen profile during extract treatment [12]. To investigate this possibility, we evaluated its estrogenic/antiestrogenic potential in the ovarioctomized rats. Our results demonstrated a strong estrogenic effect, and the levels of serum estrogens were found to be significantly (p<0.001) higher than control animals. Interestingly, we did not observe any significant difference in the serum estrogen levels when control+EE animals were compared with the 125 mg/kg extract+EE and 250 mg/kg extract+EE-treated animals. To add further, there was an insignificant difference in the serum estrogen levels between different doses of extract treatment, in both EE-treated and non-treated groups. These results suggest that the estrogenic effect of T. govanianum is a saturable process. This may be explained on the basis of steroidal phytochemical, trillian, present in this plant [4], which can be converted into bioactive steroid diosgenin within the body through hydrolysis, which is a saturable process. Moreover, this gives an added advantage for the anti-implantation effect of this plant in the clinical settings, as the estrogen levels will not increase uncontrolled and therefore side effects of antifertility agents such as hormonal imbalance could be eliminated. Our results are in agreement with the previous reports where several plant extracts, namely Afromosia laxiflora, Pterocarpus erinaceus, Michelia champaca, and Strigo orbobachioides, have been reported to possess

**Table 1: Anti-implantation and estrogenic/antiestrogenic effect of the hydroalcoholic rhizome extract of T. govanianum**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Anti-implantation study</th>
<th>Estrogenic/antiestrogenic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% change in body weight</td>
<td>Number of implantation sites</td>
</tr>
<tr>
<td>Control</td>
<td>5.48±0.01</td>
<td>7.8±0.84</td>
</tr>
<tr>
<td>EE</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T.G-125</td>
<td>3.64±0.03</td>
<td>1.4±0.55***</td>
</tr>
<tr>
<td>T.G-250</td>
<td>4.09±0.61</td>
<td>0***</td>
</tr>
<tr>
<td>T.G-125+EE</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T.G-250+EE</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

EE: 17α-ethinylestradiol (administered through subcutaneous injection at the dose of 1 μg/rat/day); TG-125: Hydroalcoholic rhizome extract of T. govanianum (administered orally at the dose of 125 mg/kg); TG-250: Hydroalcoholic rhizome extract of T. govanianum (administered orally at the dose of 250 mg/kg). *Treatment versus control: *p<0.05, **p<0.01, and ***p<0.001.
estrogenic activity [12,14], which has been associated with antifertility properties [12].

Moreover, changes in estrogen and progesterone balance may lead to abortion or termination of pregnancy, which is the mechanism used by the clinical synthetic steroidal oral contraceptives [15-17]. Rhizomes of *T. govanianum* are a rich source of steroids, which can be hydrolyzed to diosgenin [4], which is used as a raw material for manufacturing synthetic steroidal contraceptives [15]. Therefore, orally administering TGE might have disturbed estrogen-progesterone balance to produced antifertility effect.

**CONCLUSION**

Oral administration of TGE to female Wistar rats induces dose-dependent anti-implantation activity, which can be attributed to its strong estrogenic potential and uterine thickening. Estrogenic effect in ovariectomized rats was observed to be a saturable process and thereby could be free from the side effects of currently used steroidal contraceptives. *T. govanianum* could find a clinical application as a safer and efficient herbal birth control strategy.

**ACKNOWLEDGMENT**

Authors would like to acknowledge the Department of Biotechnology, Bioinformatics, and Pharmacy, JUIT, for providing all the resources to conduct this research. We would acknowledge Mr. Arun Parashar for his inputs during animal experimentation and tissue collection. We would acknowledge Nucleus Academy of Excellence, Solan, Himachal Pradesh (http://nucleas.org/), for their inputs in the manuscript preparation and editing.

**AUTHORS’ CONTRIBUTION**

Shivam Sharma contributed in experiment design, plant collection, extract preparation and characterization, manuscript preparing, and editing. Vineet Mehta designed and performed animal experiments, result analysis, histopathology, prepared and finalized manuscript. Parul Sharma and Kritika Jaggi contributed in extract preparation, characterization, and animal experiments. Dr. Udajabahu M. and Dr. Hemant Sood designed the entire study, provided necessary funding, analyzed the results, edited and finalized the manuscript.

**CONFLICTS OF INTEREST**

Authors declare no conflict of interest of any kind for the study conducted.

**REFERENCES**


