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Detection of Polycystic Ovary Syndrome and Its Treatment with *Pergularia daemia* in Rat Models

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Abstract: *Polycystic Ovary Syndrome (PCOS) is a heterogeneous, multifaceted disorder which affects mostly the women in the reproductive stage with unclear etiologies. This syndrome is associated with clinical, endocrinological and metabolic dysfunction. The ultimate aim of this research is to analyze about the estrous cycle in the PCOS induced rat models and its treatment with a common trellis vine called Pergularia daemia. Female albino wistar strain rats were given Testosterone Propionate an androgenic hormone intraperitoneally to induce PCOS. The PCOS affected rats were treated orally with the leaf extract of P. daemia. The vaginal smears were observed for a period of 15 days for three different groups namely control, PCOS induced and P.daemia treated. The result shows that irregularity of estrous cycle phase in the PCOS induced group. The remarkable changes were found in the P.daemia treated group by normalizing the estrous cycle and it is quite similar to the control. The statistical analysis also proves that there is a similarity ($P < 0.05$) between the control and the P.daemia treated group through One way ANOVA. Thus, the current study strongly proves that P.daemia is an effective drug in treating the PCOS which is a major reason for the cause of infertility.*

Key Words*:* Polycystic Ovary Syndrome, Vaginal smear, albino rats, Testosterone, *Pergularia daemia*

I. Introduction

The Polycystic Ovary Syndrome (PCOS) is the commonest endocrine disturbance mostly 4% to 12% of the women are affecting in the reproductive age [1,2]. This syndrome has the heterogenous collection of the signs and symptoms that gathered together to the form a spectrum of disorder with the mild representation in some, whilst in others a severe disturbance of reproductive, endocrine and in the metabolic function [3]. In the year 1935, two great researchers Stein and Leventhal reported about the ovarian morphology and clinical findings in women and coined the term Polycystic ovary Syndrome. Henceforth PCOS is otherwise called as Stein – Leventhal syndrome [4].

The most common features of PCOS are irregular menstrual cycles or amenorrhoea, excess amount of androgen hormone associated with the signs like hirsuitism, acne, and alopecia. Other metabolic disorders like obesity, type 2 diabetes and cardiovascular problems can also be included as its features [5].

Generally PCOS was diagnosed by the basis of clinical features of the menstrual dysfunction, obesity, infertility and the direct visualization of the ovaries at the laparotomy, but in the NIH conference agreed that biochemical markers like the LH/FSH ratio, increased testosterone hormone levels in the blood is also a diagnostic criteria for PCOS [6].

The characteristic polycystic ovarian morphology is found upto 22% of the normal population, with > 90% of these women have at least one mild symptom that may be considered as a clinical marker of PCOS [7,8]. During the reproductive years, PCOS is associated with the important reproductive morbidity, including abnormal bleeding, increased pregnancy loss and complication of pregnancy [9].

About 50% of the women are obese particularly abdominal obesity in common, suggesting that elevated androgen levels might increase the amount of adipose tissue, particularly in the abdominal region. Imbalance of hormones prevents the ovaries from releasing an egg each month. So ovulation does not occur and the ovaries produce more immature eggs. These eggs turn into cysts and the ovaries become large and studded with numerous cysts. This is called Polycystic Ovary Syndrome [10].

The pathophysiologies were reviewed by the diagnosis related to the PCOS condition. The PCOS is not only based on the reproductive health issues, but the metabolic and the cardio vascular issues are also raised [11]. In 2003, an international consensus group proposed that the diagnostic criteria for PCOS are ovarian

dysfunction as an evidenent by oligomenorrhea or amenorrhea and clinical evidence of androgen excess compared with hirsutism and acne [12,13].

Several theories are proposed to explain the pathogenesis of PCOS [14]. They are

1. An alteration in gonadotropin – releasing hormone secretion results in increased Luteinizing hormone (LH) secretion.
2. An alteration in insulin secretion and insulin action results in hyperinsulinemia and insulin resistance.
3. A defect in androgen synthesis that results in increased ovarian androgen production.

Excess secretion of LH is a hallmark study for the cause of PCOS. PCOS women have increase in LH pulse frequency and amplitude. This increased frequency results in increasing the frequency of hypothalamic gonadotropin releasing hormone (GnRH). Hypersecretion of LH results in increased production of androgen by the thecal cells in the ovaries [15,13].

The principle features of PCOS can be traced out through the concentration of the androgens like testosterone, androstenedione and the precursor of androgen Dehydroepiandrosterone sulphate (DHEA -S) while the symptoms of the hyperandrogenism may be due to inborn defects in ovarian steroidogenesis, excess steroidogenesis by the ovaries due to hyper insulinemia and the excess LH stimulation [16].

Nowadays the PCOS are interlinked with many disorders and it is not easy to diagnose the symptoms only with the help of ultrasonography but with some active measures like serum analysis for the detection of the hormonal levels. The easiest way for the detection of the PCOS condition is vaginal smear analysis. The alteration in LH brings out a serious effect in the estrus stage in rats. The estrus cycle regulation is blocked by the LH hormone synthesis in excess in the PCOS condition [17].

In ancient times there was a large of number natural products identified from the natural resources based on their traditional medicine. According to the WHO 80% of the world population consumed the herbal medicine as teas, decoctions or extracts directly or indirectly with the easily accessible liquids such as water, milk or alcohol [18].

Medicinal research has gained a renewed focus recently due to the interest of the researchers. The other prime reason is that other medicines will surely brings out the side effects but the herbal or the natural product brings zero side effects [19].

Pergularia daemia belongs to a milky weed family called Asclepiadaceae. This family includes more than 2000 species that can be classified under 280 genera which have many medicinal properties that helps to cure tooth ache, diarrhea, malarial fever and diabetes. The plant is distributed worldwide in the tropical and the sub-tropical regions [20].

Nowadays people are rushing towards allopathic medical centers get the temporary remedy for many diseases. Similarly Metformin is an agmark allopathic medicine for PCOS, Hypothyroidism and diabetes. Some of the herbal medicines like spearmint tea are also given to PCOS patients. Thus the ultimate goal of the current study is to test out the efficacy of the *Pergularia daemia* plant for the treatment of PCOS in rat models through vaginal smear technique.

II. Materials and Methods

2.1. Experimental animal

Female Albino Wistar strain rats (*Rattus norvegicus*) were used as an experimental animal with the body weight 150 – 200 grams purchased from King's Institute Chennai. The animals were acclimatized to the laboratory conditions for 15 days fed with pellet and water ad libitum, pellets were purchased from Sai Durga Enterprise. After the acclimatization period the rats were used for the experimental work. Institutional Animal Ethical Committee approvals were taken prior to perform this study (437/01/c/CPCSEA) (Ref. 01/2011).

2.2. Induction of PCOS in rats

2.3. Stock Preparation

2.5 grams of Testosterone Propionate (TP) a powdered male hormone were purchased from Himedia Company (Mumbai) and were dissolved in 50 ml of olive oil which is a solvent

2.4. Experimental Design

Three different groups of animals were taken Group I as control receiving only olive oil injected intraperitoneally (ip) to test the efficacy of the solvent, Group II and Group III were injected with testosterone propionate (400mg concentration) over a period of seven days. After a week the Group III were treated with fresh crude extract of *Pergularia daemia* over a period of seven days. At the experimental period the vaginal smear were performed.

2.5. Preparation of Plant Extract

Pergularia daemia

Pergularia daemia, commonly called as Trellis – vine belongs to Apocynaceae family. It is a perennial vine found in the road sides were collected from Golden rock, Tiruchirappalli. This vine is commonly found in the tropical regions of Asia with the vernacular name "Veli -paruthi". Fresh leaves were washed, chopped and were crushed well in the Mortar and Pestle. The fresh juice (crude extract) from the leaves were collected and used for this study.

Figure 1: The aerial parts of the trellis vine – *Pergularia daemia*

CLASSIFICATION

Kingdom	:	Plantae
Order	:	Gentianales
Family	:	Apocynaceae.
Genus	:	Pergularia
Species	:	daemia

2.6. Swab Smear Technique

Moistened cotton swab were inserted into the vagina and the cells were removed from the vaginal lumen and walls. The cells were transferred on a clean glass slide. The unstained cells were observed under the microscope and photographed with the Axiostar microscope unit.

2.7. Statistical Analysis

All the data were analyzed by One – way ANOVA using Statistical Package for Social Sciences (SPSS) software (IBM Version 20).

III. Result

The present study consists of three different groups namely Group I (Control), Group II (PCOS induced) and group III (*P.daemia* treated). PCOS can be easily detected through the irregular estrous cycle in rats. The cells from the vagina bring out a clear-cut idea about the different stages of the estrous cycle. From the vaginal smear different stages were analyzed for 15 days and the results were interpreted through SPSS.

Figure 1 shows the aerial parts of the trellis vine – *Pergularia daemia.* Figure 2 a depicts the Estrous stage of control rat fully loaded with the Keratinocytes. Figure 2b shows the Metestrous stage with the small rounded cells called Leukocytes and the Cornified cells. Large number of Leukocytes alone found in the Diestrous stage and the epithelial cells are found in the Proestrous stage of the control rats (2c and 2d).

Figure 3a, 3b, 3c shows the Metestrous, Diestrous and the Proestrous stages of the PCOS induced rats in which large number of Leukocytes, Cornified cells and few number of epithelial cells were found. There is no chance of estrous appearance due to the excess amount of testosterone, the main culprit for the formation of PCOS. Figure 4a indicates the Diestrous stage loaded completely with the Leukocytes while in Figure 4b there is a change in the cells indicates the Metestrous stage with some Leukocytes and the Cornified cells. Fig 4c depicts the Proestrous stage with the epithelial cells and finally the regulation of the estrous cycle is found in the *P. daemia* treated group (4d) by the appearance of the estrous stage loaded fully with Keratinocytes. This incident shows that *P. daemia* have good effect in regularizing the estrous cycle.

Table 1 depicts about comparison of estrous cycle stages of three different groups of rats. The control rat shows a normal cycle with the series of all the stages like Estrous, Metaestrous, Diestrous and Proestrous. The Polycystic ovary syndrome induced group shows the irregularity of the menstrual cycle mostly with the Diestrous stage and the Metaestrous stage.

In the third group i.e. *P.daemia* treated group shows the reversible process of the irregular estrous cycle. This group shows the diestrous stage in the first three days after the crude extract treatment to the PCOS induced rats shows the regularized estrous cycle. This clearly shows that *P.daemia* is an effective drug in treating the PCOS condition. The estrous cycle stages of the control, PCOS induced and *P.daemia* treated group were statistically analysed through One - way ANOVA test, showing significant differences ($P < 0.05$) (Table 2). Thus we confirmed that *P. daemia* have a potential effect in treating PCOS and brings similar to that of the control (Table 3).

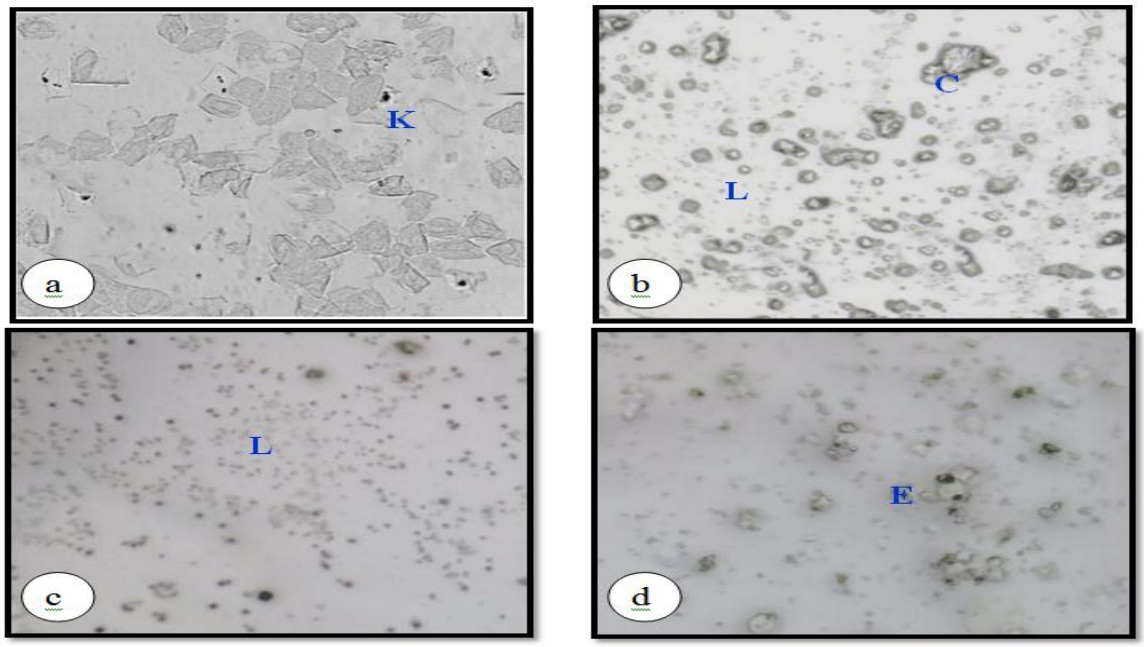

Figure – 2: Stages of Vaginal Smear for Group I (Control) 100x

a) Estrous Stage b) Metestrous Stage c) Diestrous Stage d) Proestrous Stage

K – Keratinocytes, E – Epithellial Cells, C – Cornified Cells, L – Leukocytes

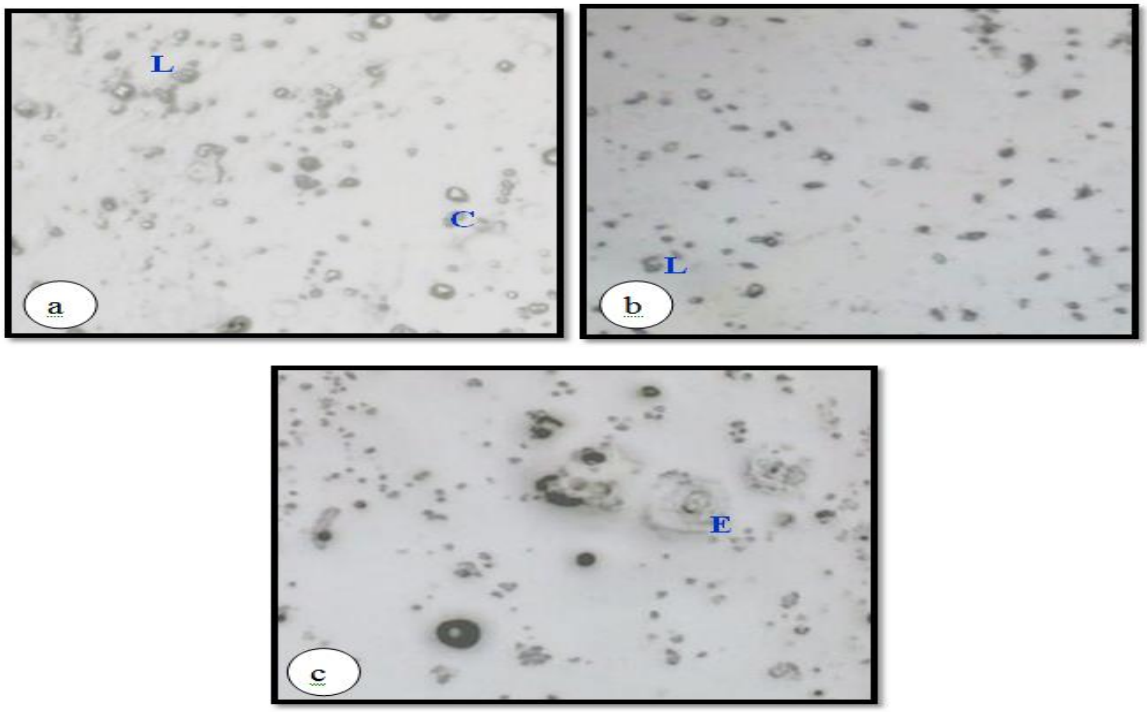

Figure – 3: Stages of Vaginal Smear for Group II (PCOS induced) 100x

a) Metestrous Stage b) Diestrous Stage c) Proestrous Stage

E – Epithellial Cells, C – Cornified Cells, L – Leukocytes

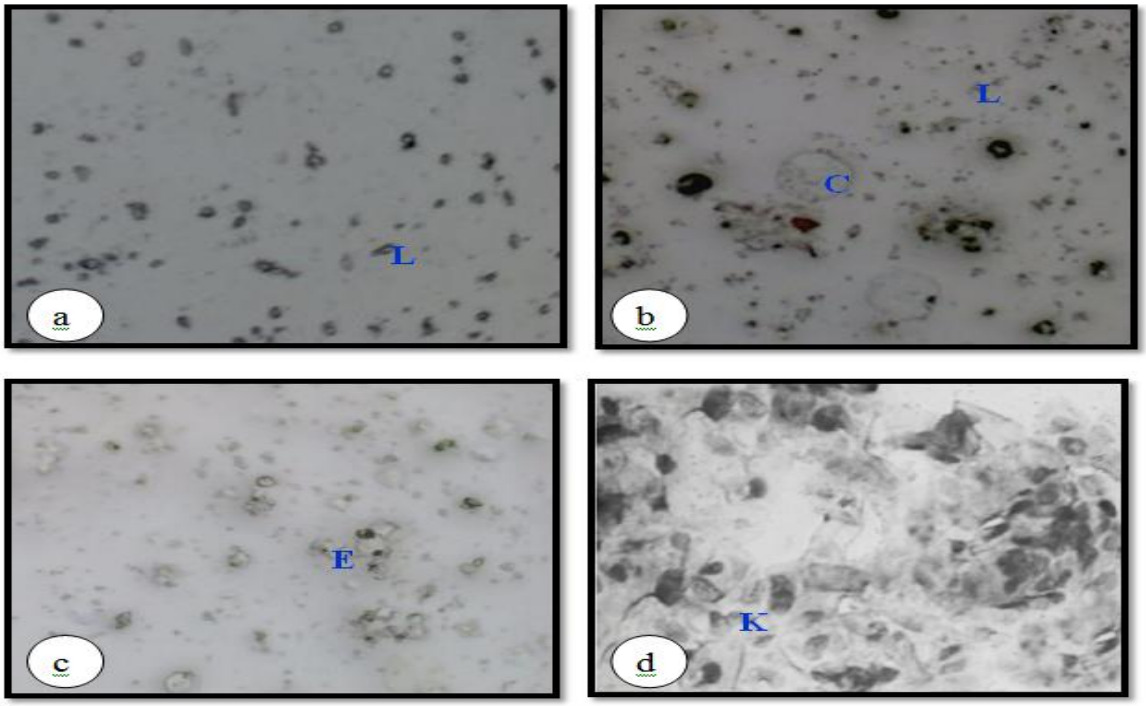

Figure – 4: Stages of Vaginal Smear for Group I (*P.daemia* treated) 100x

a) Diestrous Stage b) Metestrous Stage c) Proestrous Stage d)Estrous stage

K – Keratinocytes, E – Epithellial Cells, C – Cornified Cells, L – Leukocytes

No. of days	**Group I (Control)**	**Group II (PCOD induced)**	**Group III (*P.daemia* treated)**
1.	Estrous	Diestrous	Diestrous
2.	Estrous	Diestrous	Diestrous
3.	Estrous	Diestrous	Proestrous
4.	Metestrous	Metestrous	Metestrous
5.	Metestrous	Metestrous	Metestrous
6.	Diestrous	Proestrous	Metestrous
7.	Diestrous	Proestrous	Diestrous
8.	Proestrous	Diestrous	Proestrous
9.	Proestrous	Diestrous	Estrous
10.	Estrous	Diestrous	Estrous
11.	Estrous	Diestrous	Estrous
12.	Estrous	Diestrous	Metestrous
13.	Metestrous	Proestrous	Metestrous
14.	Metestrous	Proestrous	Metestrous
15.	Diestrous	Metestrous	Proestrous

Table 1: Estrous cycle identification and comparison of different groups of albino wistar strain rats through vaginal smear

Table 2: Statistical analysis through One - Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.600	2	3.800	4.030	.025
Within Groups	39.600	42	.943		
Total	47.200	44			

Table 3: Homogeneous Subsets of different groups

			Subset for alpha = 0.05	
	groups	N	1	2
Tukey HSDa	Control	15	2.0667	
	Pergularia daemia treated	15	2.4667	2.4667
	PCOS induced	15		3.0667
	Sig.		.502	.220
Duncana	Control	15	2.0667	
	Pergularia daemia treated	15	2.4667	2.4667
	PCOS induced	15		3.0667
	Sig.		.266	.098

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 15.000.

IV. Discussion

Polycystic ovary syndrome induction in the rat models can be performed by various methods but hormones are the only active chemical source to induce PCOS [21]. Injecting androgen to the animal is the wise and the successful method for the PCOS induction [22]. Some other techniques are also involved in the induction of PCOS. One such major technique is heat. PCOS can be induced in rats by the continuous exposure of the sunlight. This brings out the hypothalamic lesions [23]. PCOS conditions successfully obtained by inducing Testosterone Propionate in 21 days age albino rats, the ovaries shows large cystic follicles with the accumulation of multilayered immature pre – antral follicles [24]. PCOS is also induced in 21 days old rats with Letrozole compound [25].

Estradiol Valerate is an effective compound to induce PCO in rats weighing 205g to 230g [26]. This forms antral cysts in the ovaries. Testosteronant is an oil suspension injection helps to induce PCOs in 21 days old rats. This brings cystic condition in the antral follicular regions [27]. Testosterone propionate also helps in obtaining PCOS condition in the rats of 150 – 200gm body weight [28]. The testosterone administration in the female rats brings an acute effect on the glucose transport, reduced capillary density in the muscles. The androgen is partially responsible for the insulin resistance in muscle in PCOS women [29]. Preclinical investigations are found to interfere with the reproductive function of the female rat [30]. This interference is a change in normal morphology of the reproductive tract or a disturbance in the duration of particular phases of the estrous cycle. The current study supports the same statement. The changes in the estrous cycle are predominantly seen in the testosterone propionate induced group.

The rodents are the widely used animal to study PCOS, with benefits relating to their smaller size, short lifespan, high reproduction index and the different strains [31]. This statement supports the present study in which usage of rodents is highly flexible in preparing the vaginal smear due to the size and the short life span. The vaginal epithelium of the rat shows the histological variance in response to the stimulation for steroid responses [32]. The vaginal cornification with high levels of estrogens, which are characteristic of follicular cysts and it, was corroborated by the histological study. The present experiment shows that the due to the multiple follicular cysts and the vaginal smear cells may differ in its regular estrous cycle. It is due to the PCOS state. Obesity in combination with testosterone has high risk factor in causing PCOS rat models. This study is also similar to the human being who have the same metabolic and the reproductive characteristic features [33]. The present study also focuses the welfare of the human beings by treating the PCOS condition.

Several herbal therapies are available to treat PCOS condition. Epimedium herb (*Herba epimedii*) has a great effect in treating the PCOS [34]. The combination of the green tea are Dodder seed (*Semen cuscutae*) flowers of Solomonseal rhizome (*Rhizoma polygonati*), Chinese fox glove root (*Raidx rehmanniae*) and lateral roots of aconite (*Radix aconiti*) brings a drastic change in obesity and anovulation in rats that are sterilized with androgen injected with testosterone propionate [35]. The normalizing of the estrous cycle in rats with the plant celled *Tephsia Purpurea* by comparing the results with the SPSS software by performing one way ANOVA [36]. This is already proven through ovarian morphology [28]. The present study is also similar and proves that the leaves of *P.daemia* is an effective compound that brings back the reproductive cycle of the rat to the normal level. The regular cycles restored to 80 – 90 % normal level in treating with the leaf extract of *P. daemia*.

V. Conclusion

The current study is performed to detect the PCOS condition in the rat models through unstained vaginal smear technique. The disturbed estrous cycle is found in Testosterone propionate rats which indicate the major symptoms of PCOS. In Indian traditional medicine several road side plants are used to cure many complicated diseases. One such efficient plant to manage PCOS is *Pergularia daemia*. The *P. daemia* leaf extract treated rat group shows regularized pattern of estrous cycle which is similar to the control group is an authentic evidence for the recovery from PCOS. The Statistical evidence also shows significance between control and the *P. daemia* treated groups. Thus the plant *Pergularia daemia* is found to be a most effective in treating PCOS and it is an active potential herbal medicine to save the human kind from infertility.

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