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RESEARCH ARTICLE

Fertility Activity of *Wedelia trilobata* Linn. Leaf Extracts on Female Wister Albino Rats

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Abstract

Wedelia trilobata Linn., Asteraceae, is a creeping evergreen perennial herb. It is native to the tropics of Central America and has naturalized in many wet tropical and subtropics areas of the world. It is widely used in traditional medicine for the treatment of contraceptives, rheumatoid arthritis, diabetes, infertility and impotence. The present study evaluated the potential fertility effect of aqueous and ethanolic extract of leaves of *Wedelia trilobata* Linn. Anti-oxidant activity of the leaves was determined and the antimicrobial activity was performed for the urinary bacteria of females. In animal experiment, Group 1 served as negative control given only olive oil, Group 2 and 3 were given with 200 mg/kg and 400 mg/kg of Aqueous extract of *W. trilobata*, Group 4 and 5 – 3 was given with 200 mg/kg and 400 mg/kg of ethanolic extract of *W. trilobata*, Group 6 and 7 were given 1mg/kg and 2 mg/ kg of drug. The results revealed the promising anti-oxidant and anti-microbial activity and the aqueous treated rats shown similar activity compared to the control rat. The contraceptive nature of the rats was determined through the hormonal reports.

Keywords: Anti-Microbial, Anti-Oxidant, Fertility Activity, *Wedelia trilobata* Linn, Wister Albino Rats

1. Introduction

Population explosion has created a grave setback in the economic growth and all-round human development in developing countries. Current pandemic population explosion demands an immediate betterment of new potential contraceptives[1]. Studies of many years have highlighted the unmet demand for safe, inexpensive, and acceptable contraceptives to avoid unwanted pregnancies and resultant abortions. The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although a wide variety of synthetic contraceptive agents are available, these cannot be used continuously due to their severe side effects. Hence, people are looking back to age-old tradition of using herbal medicines, which have minimum side effects[2] and *Wedelia trilobata* (Asteraceae) is one such plant. India in general and Western Ghats region in particular have enormous wealth of medicinal plants. Roots are used in leprosy, decoction of leaves is used as purgative and stomachic, leaves are used as febrifuge for intermittent fevers, and latex is used on ulcers[3]. It contains compounds other than diterpenoids. The chief compounds reported are triterpenoid, sterol, alcohol, and hydrocarbon. The phenolic compounds include flavonoid lignans, coumarin tannin, phenanthrenes, quinones, phenolic acid, alkaloids, cyanogenic glycosides, and glycosylates[4]. Literature survey revealed that no systematic approach has been made to study the reproductive toxicity of leaves of this plant. In the present work, we have investigated the reproductive toxicity of the extracts of *Wedelia trilobata* leaves.

2. Materials and Methods

2.1 Plant Collection and Extraction

The *W. trilobata* leaves were collected from Thanjavur nursing garden. Then leaves were washed with tap water

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and distilled water before initiating the experiment. 100g of *W. trilobata* (L.) leaves were weighed and extracted in Soxhlet's apparatus using Distilled water, ethanol as a solvent. Then the extract was filtered using what man No. 1 filter paper and stored at 4°C. The solvents were removed using rotary vacuum evaporator and stored in desiccator for further use[5].

2.2 DPPH Assay

The effect of ethanolic, aqueous extracts and caryophyllene compound on DPPH radical was estimated using the method of (George H *et al.*, 1996)[6]. DPPH solution was freshly prepared by dissolve 24 mg DPPH in 100 ml ethanol, stored at -20°C before use. 150 µl of samples (10 µl samples + 140 µl distilled water) is allowed to react with 2850 µl of DPPH reagent (190 µl reagent + 2660 µl distilled water) for 24 h in the dark condition. Absorbance was measured at 515 nm. Standard curve is linear between 25 to 800 µM ascorbic acid. Results expressed in µm AA/g fresh mass. Additional dilution needed if the DPPH value measured will over the linear range of the standard curve. All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to formula

% inhibition= [{Abs control - Abs sample}/Abs control] × 100

2.3 Antimicrobial Assay

Proteus mirabilis, Escherichia coli, Entercoccus faecalis, Pseudomonas aeruginosa, Klebsilla sp., *Streptococcus agalactiae, Staphylococcus aureus* were purchased from MTCC. All bacteria were grown on nutrient agar media.

2.3.1 Paper Disc Method

A swab of the bacteria suspension containing 1×10^8 cfu/ml was spread on to Petri plates containing nutrient agar media. Final concentration of the extracts were 10 mg/ml. Sterile filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37°C for 24 h. The Distilled water served as negative control while the standard streptomycin (10 µg) discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs[5].

2.4 Reproductive Activity

2.4.1 Animal

White albino rats weighing between 120 and 140 g were used. These animals were housed in stainless steel cages (six in each cage, 42 rats for total represented 7 groups) under ambient temperature of 27±2°C with 12 h light/dark schedule. They were fed with the standard commercial diet and provided with water for 7 days to be acclimatized with the hold.

2.4.2 Experimental Design

The normal rats were divided into seven groups of six rats each as follows. Group 1 served as negative control given only olive oil, Group 2 and 3 were given with 200 mg/kg and 400 mg/kg of Aqueous extract of *W. trilobata*, Group 4 and 5 – 3 was given with 200 mg/kg and 400 mg/kg of ethanolic extract of *W. trilobata*, Group 6 and 7 were given 1mg/kg and 2 mg/ kg of drug respectively.

2.4.3 Estimation of Total Protein, Cholesterol, Triglyceride

Total protein, Cholesterol and Triglyceride concentration of Serum, liver, uterus, ovary of Wister albino rat was estimated according to the method Sherif Hassan *et al.*, 2010[7].

2.4.4 Vitamin C Content

Vitamin C levels were determined calorimetrically as described by Jacques-Silva *et al.*, 2001[8].

2.4.5 Estimation of GSH and SOD

The levels of hepatic reduced glutathione (GSH) and totalthiol (T. thiol) and the activity of hepatic Superoxide Dismutase (SOD) were determined by the methods Sherif Hassan *et al.*, 2010[7].

2.4.6 Biochemical Assays

Urea, creatinine, bilirubin, Albumin, goblin and uric acid levels of Serum, liver, uterus and ovary were determined using commercial Kits.

2.4.7 Enzyme Activity

Biochemical studies were carried out using standard methods for Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, conjugated bilirubin, total protein, albumin, urea and creatinine.

2.4.8 Hematological Studies

Estimation of haemoglobin, RBC, WBC, Packed cell volume were done by the method described by Kefas M *et al.*, 2015[9].

2.4.9 Hormonal Reports

The assay for LH, FSH, Prolactin, Estrogen and Progesterone was done in accordance with established principles using appropriate hormonalkit[10].

3. Result and Discussion

3.1 Anti-oxidant Activity

Table 1 indicates scavenging activity for free radicals of DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Among five different concentration of leaf extracts of *W. trilobata*, the aqueous leaf extract of *W. trilobata* recorded the most effective DPPH radical scavenging activity 0.514± 0.006in 100 µg/ml concentration. These values are being very close to synthetic antioxidant Ascorbic as positive control.

Table 1. Anti-oxidant activity of various extract of *W. trilobata*

DPPH Radical Scavenging Ability				
Concentration	Standard (L- Ascorbic Acid)	Ethanol	Compound	Aqueous leaf
20 µg/µl	0.869± 0.004	0.881± 0.014	0.933± 0.002	0.944± 0.003
40 µg/µl	0.772± 0.004	0.75±0.007	0.816± 0.001	0.867± 0.010
60 µg/µl	0.604± 0.004	0.604± 0.002	0.702± 0.001	0.730± 0.005
80 µg/µl	0.414± 0.005	0.403± 0.002	0.536± 0.005	0.643± 0.004
100 µg/µl	0.171± 0.043	0.190± 0.007	0.318± 0.003	0.514± 0.006

3.2 Anti-microbial Activity

The antibacterial activity of aqueous extract of *Sphagneticola trilobata* was found significant against four bacteria, *Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Streptococcus agalactiae*. The aqueous extract showed very high activity in *Proteus mirabilis, Pseudomonas aeruginosa* with the zone of inhibition of 22 mm diameter in 100µg concentration. The standard (10µg streptomycin) showed zone of inhibition 16mm, 10mm, 11mm in *Proteus mirabilis Enterococcus faecalis, Klebsilla sp.* respectively. Standard shows no activity in some microbe but sample showed prominent zone of inhibition (Table 2).

Table 2. Anti-Microbial activity aqueous extract of *W. trilobata*

Organisms Name/ concentration of Extract	Zone of inhibition (mm)				
	25µg	50µg	75µg	100µg	Control (10µg streptomycin)
Proteus mirabilis	16	20	21	22	16
Escherichia coli	10	15	18	20	-
Enterococcus faecalis	13	16	17	19	10
Pseudomonas aeroginosa	11	14	18	22	-
Klebsilla sp.	10	11	12	12	11
Streptococcus agalactiae	14	16	18	20	-
Staphylococcus aureus	10	12	13	14	-

The antibacterial activity of ethanolic extract of *Sphagneticola trilobata* was found significant against two bacteria, *Entercoccus faecalis, Escherichia coli*. The ethanolic extract showed very high activity in Escherichia coli, *Entercoccus faecalis, Staphylococcus aureus, Proteus mirabilis Pseudomonas aeruginosa* with the zone of inhibition of 19mm, 19mm, 17mm, 15mm, 15mm diameter in 100µg concentration. The standard (10µg streptomycin) showed zone of inhibition 17mm, 16mm, 16mm in *Proteus mirabilis Staphylococcus aureus, Klebsilla* sp. respectively. Standard shows no activity in some microbe but sample showed prominent zone of inhibition (Table 3).

The antibacterial activity of Compound Caryophllene was found significant against all bacteria. The Caryophllene showed very high activity in *Staphylococcus aureus, Klebsilla* sp. with the zone of inhibition of 19mm diameter in 100µg concentration. The standard (10µg streptomycin) showed no zone of inhibition in *Escherichia coli* and *Pseudomonas aeruginosa* (Table 4).

Table 3. Anti-microbial activity ethanolic extract of *W. trilobata*

Organisms Name/ concentration of Extract	Zone of inhibition (mm)				
	25µg	50µg	75µg	100µg	Control (10µg streptomycin)
Proteus mirabilis	13	13	15	15	17
Escherichia coli	10	12	17	19	-
Entercoccus faecalis	14	17	19	19	-
Pseudomonas aeroginosa	11	12	13	15	-
Klebsilla sp.	12	12	13	14	16
Streptococcus agalactiae	9	13	14	11	14
Staphylococcus aureus	12	14	16	17	16

Table 4. Anti-microbial activity of compound (caryophyllene)

Organisms Name/ concentration of Extract	Zone of inhibition (mm)				
	25µg	50µg	75µg	100µg	Control (10µg streptomycin)
Proteus mirabilis	10	11	14	16	14
Escherichia coli	8	8	10	14	-
Entercoccus faecalis	11	12	12	14	10
Pseudomonas aeroginosa	10	11	16	15	-
Klebsilla sp.	16	18	19	19	15
Streptococcus agalactiae	13	14	19	15	16
Staphylococcus aureus	10	13	16	19	14

3.3 Reproductive Activity

Table 5 indicated the total protein content in the serum, liver, uterus and ovary of female rats. Serum protein was similar in Control group, Group V and VII with the value of 5.6 approx. Group IV shows elevated protein level with the value of 6.3±0.10. Control and Compound

treated group shows the similar level of protein in liver of the female rats. Group V shows slightly higher value than other groups 28.66±0.33. Uterus proteins were lowered in the compound treated groups than others. The compound and extract decreasing the protein level of the ovary in female rat.

Table 5. Effect on *Wedelia trilobata* Linn. leave extract on total proteins in serum, ovary, uterus and liver

S. No	Extract Administration	Total Protein			
		Serum (g/dl)	Liver (mg/g)	Uterus (mg/g)	Ovary (mg/g)
1	Group – I	5.6±0.37	27.66±0.33	20.2±0.86	35.16±2.89
2	Group – II	5.8±1.02	26.66±0.33	15.5±0.63	19.98±3.80
3	Group – III	5.7±0.08	28.66±0.66	21.47±0.72	23.33±0.98
4	Group – IV	6.3±0.10	23.43±0.72	7.23±0.46	14.4±0.60
5	Group – V	5.6±0.71	28.66±0.33	18.38±0.56	12.99±1.60
6	Group – VI	5.8±0.17	24.66±0.88	11.76±0.86	12.93±2.00
7	Group – VII	5.6±0.03	27.2±0.41	9.53±0.78	13.26±0.95

Table 6 indicated the total cholesterol content in the serum, liver, uterus and ovary of female rats. Serum protein was similar in Control group, Group III, Group V and VII with the value of 5.6 approx. Group IV shows elevated cholesterol level with the value of 6.3±0.10. Control and Compound treated group shows the similar level of cholesterol in liver of the female rats. Uterus cholesterol were higher in the compound treated groups than others. The compound and extract increasing the cholesterol level of the ovary in female rat.

Table 6. Effect on *Wedelia trilobata* Linn. leave extract on total cholesterol in serum, ovary, uterus and liver

S. No	Extract Administration	Total cholesterol			
		Serum (g/dl)	Liver (mg/g)	Uterus (mg/g)	Ovary (mg/g)
1	Group – I	136.33±5.81	6.2±0.25	9.28±0.21	3.22±0.45
2	Group – II	245±2.88	1.52±0.13	9.56±0.29	4.71±0.19
3	Group – III	364±6.92	1.96±0.06	15.2±0.73	8.62±0.32
4	Group – IV	270±5.77	1.58±0.24	12.27±0.54	6.76±0.57
5	Group – V	366.66±24.03	1.41±0.20	11.33±0.21	13.99±0.97
6	Group – VI	279.33±9.26	1.46±0.31	7.47±0.60	5.20±0.38
7	Group – VII	413.33±7.05	1.76±0.04	14.3±1.02	12.32±0.19

Table 7 shows the Phospholipids profile of 7 groups of rats (Serum, liver, uterus, ovary) which revealed that control treated group were normal in their Phospholipids level conversely the other groups showcased the higher Phospholipids profile. Serum Phospholipids profile shows the Group III (400mg/kg BW aqueous extract treated) having higher Phospholipids than Group V (400mg/kg BW ethanol extract treated) and (Group VII 4mg/kg BW compound treated). The above results revealed that the Group III rats possess the best profile of Phospholipids.

Table 8 shows the triglyceride profile of 7 groups of rats (Serum, liver, uterus, ovary) which revealed that control treated group were normal in their triglyceride level conversely the other groups showcased the higher triglyceride level. Serum triglyceride level shows the Group III (400mg/kg BW aqueous extract treated) having higher HDL than Group V (400mg/kg BW ethanol extract treated) and (Group VII 4mg/kg BW compound treated). The above results revealed that the Group III rats possess the best triglyceride levels.

Table 7. Effect on *Wedelia trilobata* Linn. leave extract on phospholipids in serum, ovary, uterus and liver

S. No	Extract Administration	Phospholipids			
		Serum (g/dl)	Liver (mg/g)	Uterus (mg/g)	Ovary (mg/g)
1	Group – I	0.90±0.06	0.55±0.06	0.88±0.06	0.87±0.06
2	Group – II	2.35±0.46	0.59±0.04	3.55±0.46	5.53±0.29
3	Group – III	2.5±0.39	3.46±0.0	2.4±0.39	3.01±0.19
4	Group – IV	2.38±0.42	0.61±0.08	2.68±0.42	1.85±0.31
5	Group – V	2.53±0.17	0.49±0.07	1.23±0.17	1.36±0.08
6	Group – VI	3.10±0.11	0.62±0.02	3.18±0.11	3.37±0.17
7	Group – VII	3.75±0.31	0.52±0.03	4.75±0.31	2.26±0.1

Table 8. Effect on *Wedelia trilobata* Linn. leave extract on triglycerides in serum, ovary, uterus and liver

S. No	Extract Administration	Triglycerides			
		Serum (g/dl)	Liver (mg/g)	Uterus (mg/g)	Ovary (mg/g)
1	Group – I	43.66±2.72	1.01±0.07	0.88±0.05	3.50±0.13
2	Group – II	95±2.88	0.81±0.04	3.12±0.17	8.05±0.36
3	Group – III	67±4.72	1.42±0.09	4.72±0.26	6.48±0.48
4	Group – IV	105.66±1.85	1.06±0.20	1.39±0.29	6.65±1.66
5	Group – V	105.66±1.73	0.94±0.09	2.89±0.05	4.9±1.14
6	Group – VI	147.66±1.20	1.06±0.05	1.39±0.11	14.5±0.84
7	Group – VII	120.36±7.21	0.89±0.04	7.77±0.72	21.85±1.10

Table 9 shows the HDL profile of 7 groups of rats (Serum, liver, uterus, ovary) which revealed that control treated group were normal in their HDL level conversely the other groups showcased the higher HDL profile. Serum HDL profile shows the Group III (400mg/kg BW aqueous extract treated) having higher HDL than Group V (400mg/kg BW ethanol extract treated) and (Group VII 4mg/kg BW compound treated). The above results revealed that the Group III rats posses the best profile of HDL.

The GSH level of the uterus and ovary treated with two extracts were compared in the above table which shows higher level of Vitamin C in ovary and uterus. Elevated level of vitamin C was shown in Group III (400mg/kg BW aqueous extract treated), Group V (400mg/kg BW ethanol extract treated) and (Group VII 4mg/kg BW compound treated). Table 10 shows the contraceptive nature of Group III rats.

Table 9. Effect on *Wedelia trilobata* Linn. leave extract on HDL in serum, ovary, uterus and liver

HDL	Extract Administration	HDL			
		Serum (g/dl)	Liver (mg/g)	Uterus (mg/g)	Ovary (mg/g)
1	Group – I	66±2.08	3.11±0.28	1.17±0.15	8.80±0.46
2	Group – II	185±2.88	1.19±0.04	4.96±0.31	17.33±4.66
3	Group – III	246.66±4.63	1.06±0.14	0.66±0.16	14.68±1.74
4	Group – IV	135.66±2.96	0.48±0.04	3.77±0.07	5.82±0.50
5	Group – V	188±5.45	1.84±0.62	1.95±0.15	3.41±0.75
6	Group – VI	105.66±2.96	1.94±0.47	5.14±0.74	7.51±0.48
7	Group – VII	136.33±4.63	1.28±0.12	4.16±0.34	4.25±0.14

Table 10. Effect on *Wedelia trilobata* Linn. leave extract on Vitamin C in ovary and uterus

S. No	Extract Administration	Vitamin C	
		Uterus (mg/g)	Ovary (mg/g)
1	Group – I	16.19±2.31	47.33±2.48
2	Group – II	15.41±2.21	65±5.0
3	Group – III	41.64±11.17	21.41±4.28
4	Group – IV	61.60±9.49	43±16.09
5	Group – V	22.55±5.21	42.35±1.26
6	Group – VI	77±2.51	48.81±2.59
7	Group – VII	130±5.77	63.32±3.33

The GSH level of the uterus and ovary treated with two extracts were compared in the above table which shows heightened values on both Compound treated groups compared with control group. The GSH of ovary of Control group and Group V 2.81±0.01(400mg/kg BW ethanol extract treated)and Group III 3.35±1.11 (400mg/kg BW aqueous extract treated) were slightly different. Conversely GSH of Group V and Group III uterus shows higher value 3.77±0.16 and 5.01±1.01 than control group respectively (Table 11).

The SOD level of the uterus and ovary treated with two extracts were compared in the above table which shows the higher level of SOD were observed in the 2 mg and 4mg compound treated group. Similarly, the Group III (400mg/kg BW aqueous extract treated) shows higher value of SOD in both ovary and uterus. The level of SOD in uterus and ovary were increasing as per the increasing concentration. The SOD level was higher in all groups than control treated group (Table 12).

Table 11. Effect on *Wedelia trilobata* Linn. leave extract on GSH in ovary and uterus

S. No	Extract Administration	GSH	
		Uterus (mg/g)	Ovary (mg/g)
1	Group – I	1.96±0.05	2.85±0.14
2	Group – II	2.66±0.19	7±0.25
3	Group – III	5.01±1.01	3.35±1.11
4	Group – IV	1.47±0.05	3.25±1.03
5	Group – V	3.77±0.16	2.81±0.01
6	Group – VI	9.36±0.28	3.28±0.32
7	Group – VII	2.40±0.22	4.46±0.47

Table 12. Effect on *Wedelia trilobata* Linn. leave extract on SOD in ovary and uterus

S. No	Extract Administration	SOD	
		Uterus (mg/g)	Ovary (mg/g)
1	Group – I	559.75±28.45	548.34±8.56
2	Group – II	718.84±23.37	1327.13±173.01
3	Group – III	1316.8±90.08	782.06±34.43
4	Group – IV	1394.52±231.45	1416.66±29.62
5	Group – V	493.59±18.17	341.27±55.26
6	Group – VI	1366±88.45	532.28±30.61
7	Group – VII	1036.66±76.80	815.01±26.88

Table 13. Effect on *Wedelia trilobata* Linn. leave extract on haematological parameters

Hematological parameters	Extract Administration						
	Group – I Control (Vehicle treated)	Group – II 200mg/ kg body weight (Aqueous extract treated)	Group – III 400 mg/kg body weight (Aqueous extract treated)	Group – IV 200 mg/kg body weight (Ethanol extract treated)	Group – V 400 mg/kg body weight (Ethanol extract treated)	Group – VI 2 mg/ kg body weight (Bioactive compound treated)	Group – VI 4 mg/ kg body weight (Bioactive compound treated)
WBC (X10^9/L)	19.33±3.19	10.53±2.48	8.8±0.66	9.66±0.20	7.86±0.93	10.76±0.93	15.13±1.14
RBC (X10^12/L)	9.32±0.11	5.63±0.63	7.06±0.20	7.24±0.60	9.31±0.38	7.39±0.20	6.70±0.06
PLT (X10^9/L)	387.33±1.44	242±14.14	267.33±4.84	206.33±3.17	279±1.73	262.33±5.23	237.33±3.28
HGB (g/dl)	22.6±1.44	12.46±1.32	16.76±0.92	11.66±0.90	20.33±0.77	16.56±0.57	13.96±0.78

Table 14. Effect on *Wedelia trilobata* Linn. leave extract on enzyme activity

Enzyme activity	Extract Administration						
	Group – I Control (Vehicle treated)	Group – II 200mg/ kg body weight (Aqueous extract treated)	Group – III 400 mg/kg body weight (Aqueous extract treated)	Group – IV 200 mg/kg body weight (Ethanol extract treated)	Group – V 400 mg/kg body weight (Ethanol extract treated)	Group – VI 2 mg/ kg body weight (Bioactive compound treated)	Group – VI 4 mg/ kg body weight (Bioactive compound treated)
AST (U/L)	16±2.08	38.33±1.20	21±2.64	23.33±0.88	18.33±1.76	24±1.15	13±0.288
ALP (U/L)	116.75±4.35	122±2.0	98.15±9.81	285.15±1.3	105.41±2.20	121.50±2.20	112.48±4.75
ALT (U/L)	71.97±3.32	88.81±2.42	73±1.73	72.66±1.45	72.05±3.34	80±2.30	83.4±6.50

Table 15. Effect on *Wedelia trilobata* Linn. leave extract on organ weight

0rgan weight (g)	Extract Administration						
	Group – I Control (Vehicle treated)	Group – II 200mg/ kg body weight (Aqueous extract treated)	Group – III 400 mg/kg body weight (Aqueous extract treated)	Group – IV 200 mg/kg body weight (Ethanol extract treated)	Group – V 400 mg/kg body weight (Ethanol extract treated)	Group – VI 2 mg/ kg body weight (Bioactive compound treated)	Group – VI 4 mg/ kg body weight (Bioactive compound treated)
Liver	7.94±0.19	6.96±0.13	4.08±0.44	5.61±1.17	5.08±0.46	6.69±0.82	6.30±0.50
Kidney	1.86±0.10	1.31±0.18	1.45±0.17	1.66±0.22	1.44±0.05	1.58±0.21	1.46±0.12
Ovary	0.20±0.03	0.10±0.00	0.13±0.01	0.10±0.01	0.33±0.02	0.19±0.04	0.15±0.02
Uterus	0.86±0.04	0.59±0.18	0.48±0.16	0.46±0.06	0.97±0.10	0.75±0.24	±0.33±0.07

Table 16. Effect on *Wedelia trilobata* Linn. leave extract on hormonals activity

Hormonal reports	Extract Administration						
	Group – I Control	Group – II	Group – III	Group – IV	Group – V	Group – VI	Group – VII
LH (mlu/ml)	0.05±0.11	0.71±0.10	2.23±0.28	0.69±0.02	2.12±0.22	2.27±0.5	2.31±0.31
FSH (mlu/ml)	0.03±0.88	1.45±0.02	2.16±0.21	1.32±0.88	2.10±0.59	2.18±0.10	2.21±0.23
Prolactin (ng/ml)	0.28±0.03	0.41±0.02	0.14±0.02	0.06±0.02	0.07±0.02	0.12±0.02	0.14±0.02
Estrogen (pg/ml)	40.33±0.88	35.33±0.88	16.33±2.33	41.66±2.18	35±1.15	27.33±1.45	0.14±2.08
Progesterone (ng/ml)	10.48±0.88	5.60±1.08	11.85±0.17	5.05±0.59	16.4±0.50	13.57±0.50	19.08±0.80

The biochemical Parameters during the experimental period in Wister albino rats the RBC of female rat was very low compared to all the groups and the erythrocytes of group I (Control) and group VI (standard) was differed slightly. The second parameter WBC was higher in the Control group and lower in the Group IV compared to all groups. The Haemoglobin level of group V, VI was similar and lower than a Control group. The blood glucose level was very higher in control rat conversely very lower in other groups (Table 13).

Hepatic enzymes level during the experimental period in Wister albino rats, the comparison of AST, ALP, ALT, were done. On that group, IV shows the higher level of hepatic enzymes. The standard treated group had better results than extract and compound treated groups (Table 14).

Table 15 indicates the weight changes in liver, kidney, ovary and uterus during the experimental period in Wister albino rats. Control rats, and Groups III and V exhibited a significantly high gain in body weight and growth rate throughout the period of experiment as compared to Groups II, IV, VI. The better gain in weight was shown in Group III as compared to the Group VI and Group I with the difference of 2% approximately (Table 15).

The Table 16 indicates the hormonal assays of seven group of rats which shows the lowered estrogen level in Group VII (4mg/kg BW). When comparing the Group III and Group V, Group III shows very low estrogen level 16.33±2.33 this was the key point for the contraceptive activity. Estrogen level can determine the fertility in the females. Similarly, the progesterone level of Group VII was very higher than other groups (Table 16).

4. Conclusion

When comparing the results of lipid, cholesterol, triglycerides, HDL the aqueous extract shows promising result than ethanolic extract and compound. However, an elevation in total lipids, cholesterol and triglycerides which are well known as risk factors for cardiovascular diseases were also recorded. The aqueous extract shows the lowered level of enzymes and biochemical parameters. It could be concluded that aqueous extract of *W. trilobata* can be used as a herbal contraceptive drug that can increase the estrogen level due to its phytoestrogen components such as beta sitosterol and without affecting the effects on the other organs (liver and kidney, uterus and ovary). The above result revealed that the aqueous extract of *W. trilobata* shows the significant result compared to the control group and act as herbal contraceptives against the female rats. Further studies to evaluate sub-acute and chronic effect of this extract are recommended.

5. References

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