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The Ameliorative Effects of *Persia americana* Pulps Alcoholic Extract on Fertility of Female Rats Treated with Cyclophosphoamide

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Abstract
The experiment was designed to study *Persea americana* extract effect on female rats fertility treated with cyclophosphamide (C.P.). Plant were extracted with 70% ethanol. Rats were distributed into five groups, first group (T1) was control, second group (T2) was treated with C.P. (2mg/kg) and distilled water, third group (T3) was treated with extract (100mg /kg) and C.P, fourth group (T4) was treated with extract (200 mg/kg) and C.P, fifth group (T5) was treated with extract (300 mg/kg) and C.P. Oral administration of cyclophosphamide (2mg/kg), cyclophosphamide and *persea americana* extract (100mg/kg), to rats caused prolong the length of hours in proestrus, metaestrus and diestrus phases, in addition to prolong in days of estrous cycle when likened with T1 group, furthermore, to gradual significant reduction in hours of estrus phase as likened T1 group and further groups. The results of length estrous cycle in days and proestrus, metaestrus and diestrus phases in hours of rats in T4 and T5 groups produced a significant reduced when likened with T2 and T3 groups . Furthermore, there was no significant difference among T4 , T5 and T1 groups in all cycles. In addition, the length of estrus phase hours revealed an important surge in T4 and T5 as likened with T2and T3 groups in all cycles. This study established that extracts at doses (200mg/kg and 300mg/kg) have more defense consequence on fertility than dose (100mg/kg) of extract aganist to cyclophosphamide adverse effect .
Keywords: estrous cycle, cyclophosphamide, *Persia americana*, fertility.

1. Introduction

There are mounting interested across medicinal plants and their sprightly ingredients in the last years , the usage of herbals medicine lasts to enlarge quickly crossways the world with several of these produces used for treating of many health contests in difference state healthcare situations [1]. *Persea americana* is one of the most important herbal plant that have therapeutics benefit because it height contents of monounsaturated fat, the mainly components of the fatty oils are hydrocarbon and glycerides of oleics acid [2]. *Persea americana* pulps have large quantity of folic acid which endangered the fertility and supported reproduction, also the plant have potassium, vitamin C and Vitamin B6 which had an important role in sex hormone regulation [3]. Alkylating agents mostly cyclophosphamide has been usually correlated with sterility, the alkylating agents cyclophosphamide caused ovarian defect and gonads abnormalities, animals that received chemotherapy may experience both short and long-term ovarian damage that can be resulted in a narrowing of their reproductive window. Temporary amenorrhea is a common side- effect that immediately follows chemotherapy treatment [4]. This experiment was involved to revision the favorable effect of *Persea americana* extract on estrous cycle and fertility of rats handled with cyclophosphamide.

2. Material and Methods

2.1 Extraction of *Persea amrecana*: *Persea amrecana* pulps, cleaned and cut to small pieces then dried at room temperature. plant was crushed and powered by electrical blender and sieved, the extract was prepared by soxhlet apparatus, the pulps extract was evaporated the ethanol from it by rotary evaporators with 50 C° for four hours [5].

2.2 Experiment of Animals: mature female rats were their weight nearby 250 gram. Rats were earmarked under proper environment situations of (20-25C°) and light periods of twelve hours every day. The animals were housed in plastic cages. Foods were presumed as pellets. Provision was reserved to avoid any stupid pressure. The cages were gutted once in each week.

2.3 Experimental Design: The dual ordinary estrous cycles of fifty female rats were measured by inspection of animals vaginal smears for period of 2 weeks previously treatment. Fifty females rats were alienated into five groups, and the periods of treatment was 30 days: The rats of the initial group (T1) not given any treatment**,** the rats in second group (T2) were preserved with cyclophosphamide 2mg/kg B.W. and distilled water given orally, the rats in third group (T3) were preserved with (100mg /kg) of *Persea amrecana* extract and cyclophosphamide 2mg/kg B.W. given orally, the rats in fourth group (T4) were preserved with (200 mg/kg) B.W of *Persea*

amrecana extract and cyclophosphamide (2mg/kg) B.W given orally, the rats of the fifth group (T5) were preserved (300mg/kg) of *Persea amrecana* extract and cyclophosphamide 2mg/kg B.W given orally.

2.4 Estrous cycle detection and the periods length of estrous cycle with its phases determination: Vaginal swaps have been tested from all treated groups, the test was e each 12 hrs daily , the smear was done by interleaving plastic micro pipettes confined 0.2 ml of standard saline (NaCl 0.9%) into the vagina and the smear placed on slide of microscope (figure 1), the swaps on the slides were dried at air of room temperature and marked by metylene blue, then place the slides under microscope to categorize it into one of four phases of estrous cycle rendering to the cells types and forms, swap including nearly exclusively of leukocytes found in diestrus. cycles in all groups were inspected and the length of hours of estrous cycle phase with numbers of days have been clarified by [6], as the follows:

Hours length of phases in estrous cycle = whole hours of every phase **/** whole numbers of rats
Days length of estrous cycles = Hours length of phase in estrous cycle / 24 hr.

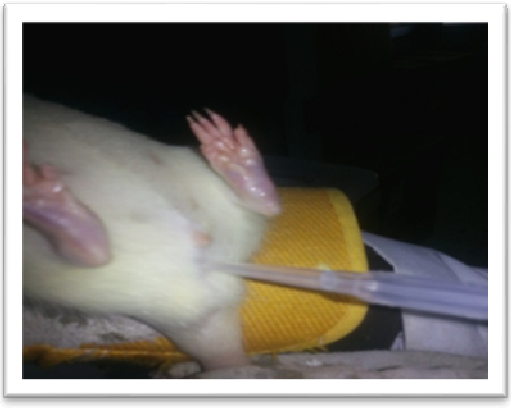


Figure (1): Vagina smears taken from female rats.

2.5 Statistical analysis: SAS [7] is a Statistical Analysis System was expressed to know the different factors in parameters of this experiment. Least significant differences (LSD) test at (P<0.05) was charity to chief compare amid averages in this experiment.

3. Results

3.1 Detection of Estrous cycles: the consequences of two cycles of estrous recognition of fifty rats previously beginning the experiment were presented in (figure 2), the metaestrus, proestrus, diestrus and estrus phases in first cycle displayed no significant difference (P<0.05) as compared with metaestrus, proestrus, diestrus and estrus phases in second cycle respectively.

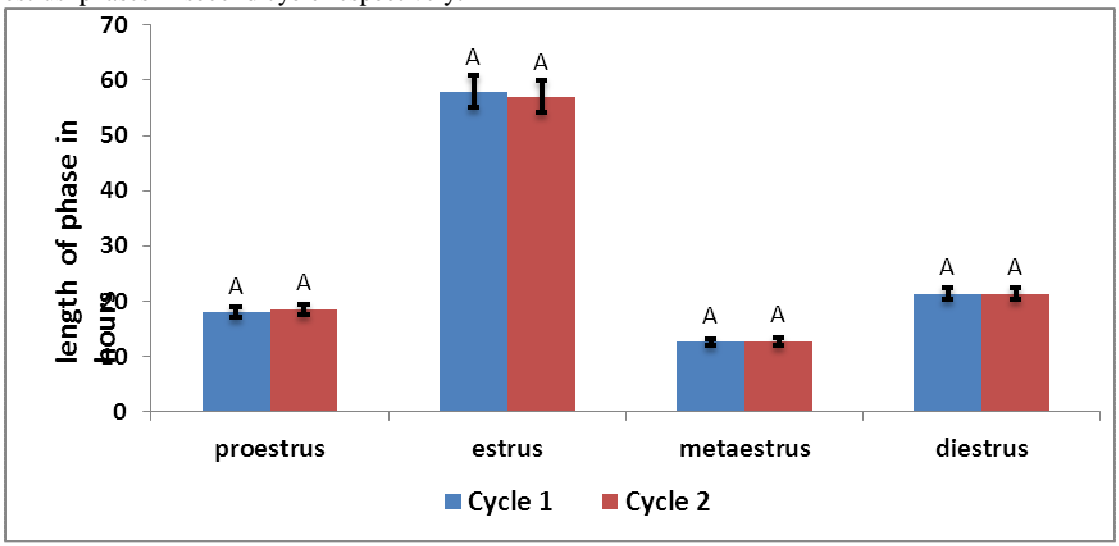
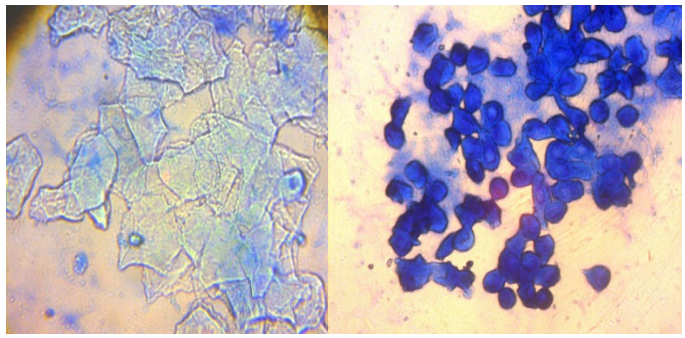


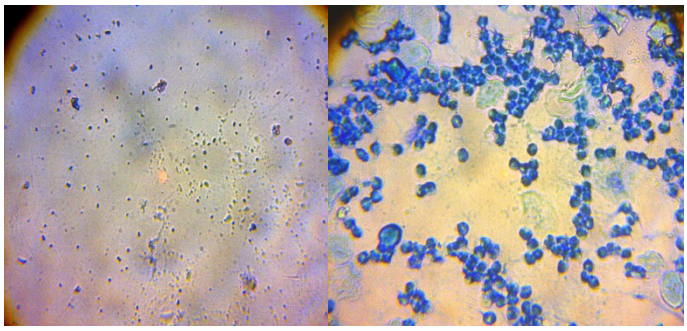
Figure (2): illustrated two estrous cycles of fifty female rats previously starting the experiment.

Furthermore, the outcomes of phases Photomicrograph exhibited in(figure3), the proestrus phase below light microscope indicated prevalence of nucleated epithelium cell and some cornifing cells seemed, whilst the estrus phase categorized by cornifing squamous epithelium cell, which arisen in clusters. There was combination of cell

kinds with a prevalence of leucocytes, a little nucleated epithelium and cornifing squamous epithelium cell in metaestrus phase, whilst in diestrus phase revealed reliable leukocytes.



A B



C D

A-proestrus B- estrus C- metaestrus D- diestrus

Figure (3): The Photographs of marked secretion of vagina from rats as in (A) represented the pro-estrus, mainly containing of nucleated epithelium cell; (B) represented the estrus, with nucleated cornifing cells; while the (C) represented metestrus, which containing of the three varieties of cells, nucleated epithelium, cornifing and leukocytes cells; and finally the (D) represented diestrus phase, containing mainly of leucocytes.

3.2 Effect all treatment on hours length of estrous phases and days length of estrous cycle: The consequences of the this research showed that oral administration of distilled water and cyclophosphamide (2mg/kg), cyclophosphamide and *Persea americana* extract (100mg/kg) to rats caused a gradual significant surge ($P<0.05$) in hour of pro estrus phase within groups until reached to mean value (30.56 ±1.24, 25.29 ±1.37) respectively in cycle five as likened with baseline group, furthermore, to significantly augmented ($P<0.05$) in all cycles as likened with other groups. while the oral administration of cyclophosphamide (2mg/kg) and *Persea americana* extracts at doses (200 and 300) mg/kg to rats produced a gradual significant reduction ($P<0.05$) in hours of pro estrus phase until reached to mean value (14.11 ±1.72, 14.00 ±1.30) respectively in cycle five as likened with cyclophosphamide treated groups, furthermore to , there was no important variance ($P<0.05$) in all cycles as likened with baseline group, as in figure (4).

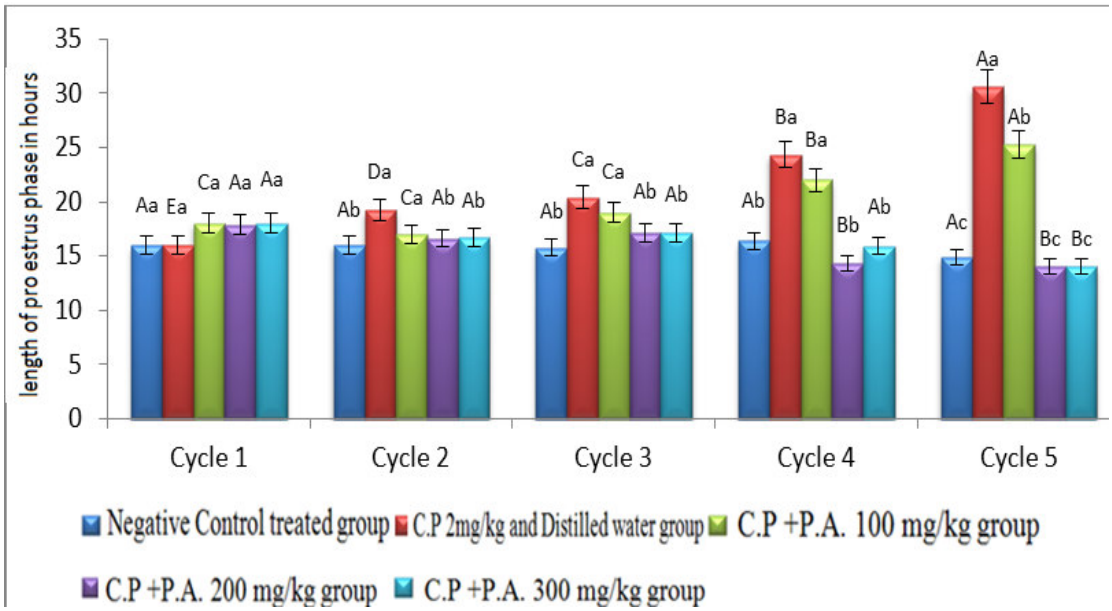


Figure (4): Effect of cyclophosphamide 2mg/kg, cyclophosphamide *and Persea americana* extract (100, 200 and 300)mg/kg and distilled water on pro estrus phase of five estrous cycles in hours. * Various capital letters referred to significantly variance (P< 0.05) between groups. * Various small letters referred to significantly variance (P< 0.05) within groups. *C.P. *: Cyclophosphamide *PA: Persea americana*

The mean values of estrus phase hours in different groups were clarified in figure (5). The hours of estrus phase of distilled water and cyclophosphamide treated group displayed a gradual significant reduction (P<0.05) until reached to average value (6.01 ±1.13) in cycle five, as likened with baseline group and further groups. While the oral administration of *Persea americana* extract (100mg/kg) caused a gradual significant decrease (P<0.05) until reached to mean value (25.81 ±1.48) in estrus phase of cycle five as compared with control group and cyclophosphamide with *Persea americana* extracts (200 and 300)mg/kg treated groups and statistically augmented (P<0.05) as likened with cyclophosphamide treated assembly. Hours of estrus phase in cyclophosphamide and *Persea americana* extracts at doses (200 and 300)mg/kg groups displayed an important increase (P<0.05), in all cycles, as likened with cyclophosphamide and *Persea americana* extract (100mg/kg) treated group and cyclophosphamide treated group , in addition there was no significant difference (P<0.05) among cyclophosphamide with *Persea americana* extract (200 mg/kg) treated group , cyclophosphamide with *Persea americana* extract (300 mg/kg) treated group and control group in all cycles.

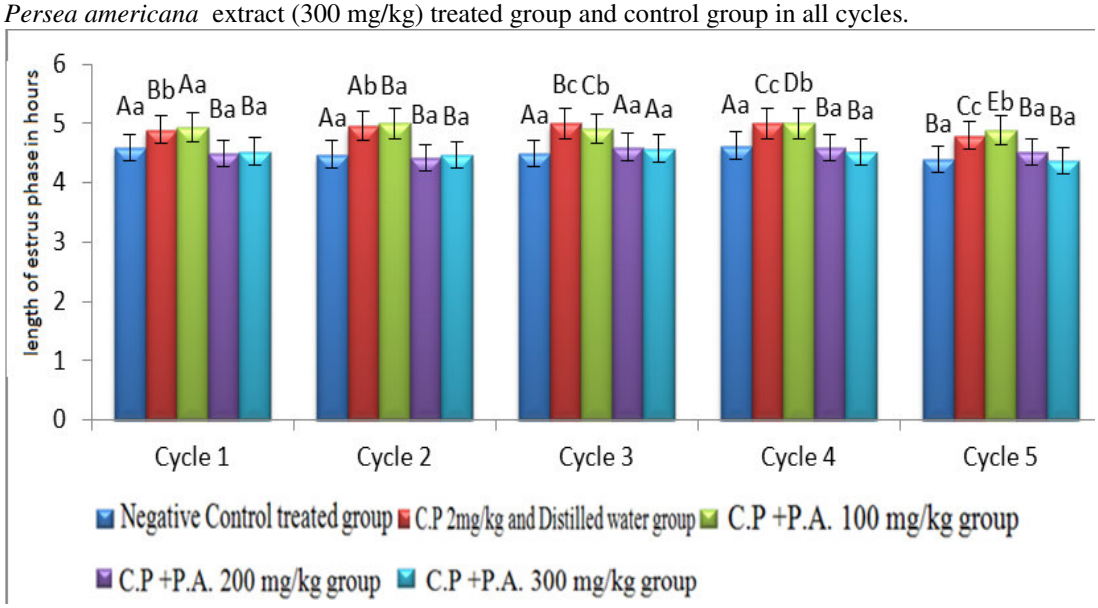


Figure (5): Effect of cyclophosphamide (2mg/kg), cyclophosphamide (2mg/kg) and *Persea americana* extracts (100, 200 and 300)mg/kg and distilled water on estrus phase of five estrous cycles in hours. *Various capital letters referred to significantly variance (P<0.05) between groups. *Various small letters referred to significantly variance (P<0.05) within groups. *C.P. *:* Cyclophosphamide *PA: *Persea americana*

The hours of metaestrus phase in distilled water and cyclophosphamide treated group were gradually and significantly increased (P<0.05) until reached to mean value (34.91 ±1.930) in cycle five, as compared with control group and others groups, while the oral administration of cyclophosphamide and *Persea Americana* extract (100mg/kg) caused a gradual significant increase (P<0.05) until reached to mean value(30.14 ±1.38) in cycle five as compared with control group and cyclophosphamide with *Persea americana* extracts (200 and 300) mg/kg groups in all cycles, in addition to significantly decreased (P<0.05) as likened with cyclophosphamide treated groups in all cycles except in cycle one. The hours of metaestrus phase in cyclophosphamide and *Persea americana* extracts at doses (200, 300)mg/kg preserved groups displayed a significantly decrease (P<0.05) in all cycles as likened with cyclophosphamide treated groups, in addition to significantly decrease (P<0.05) as likened with *Persea americana* extract (100mg/kg) and cyclophosphamide treated group in estrous cycles three, four and five. Furthermore, there was no significant difference (P<0.05) among cyclophosphamide with *Persea americana* extract (200 mg/kg) treated group , cyclophosphamide with *Persea americana* extract (300 mg/kg) treated group and control group in hours length of metaestrus all cycles, as in figure (6).

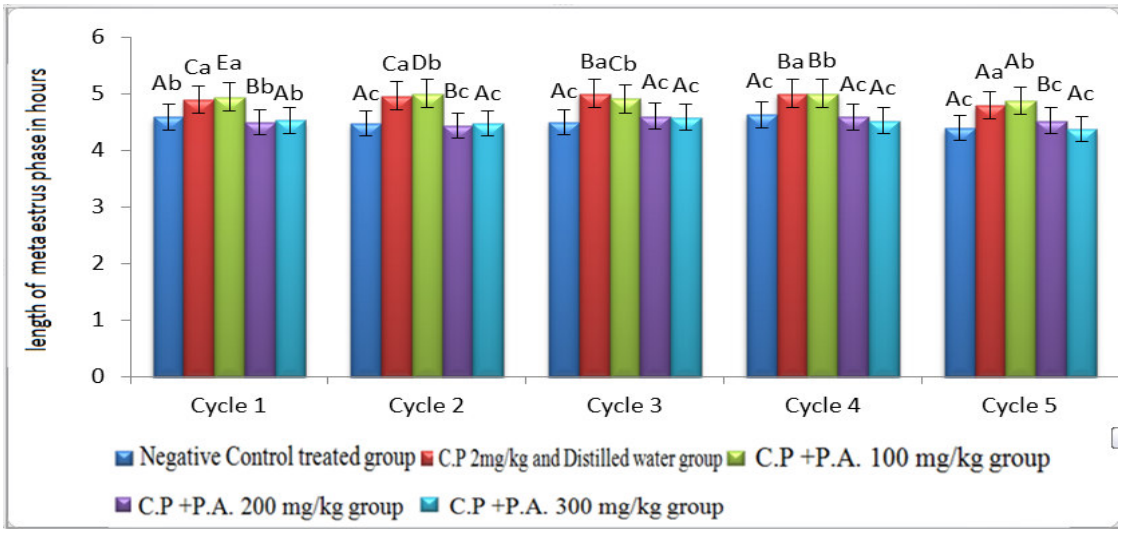


Figure (6): Effect of cyclophosphamide 2mg/kg, cyclophosphamide *and Persea americana* extract (100, 200 and 300)mg/kg and distilled water on meta estrus phase of five estrous cycles in hours. *Various capital letters referred to significantly variance (P< 0.05) between groups. *Various small letters referred to significantly variance (P< 0.05) within groups. *C.P.: Cyclophosphamide **P.A*: *Persea americana*

The hours length of diestrus phase in different groups were clarified in figure (7). The oral administration of cyclophosphamide caused gradually and important upsurge (P<0.05) in most cycles as likened with control and other groups, whereas , the oral administration of distilled water and cyclophosphamide and *Persea americana* extract (100mg/kg) caused a gradual significant surge (P<0.05) as likened with baseline group and cyclophosphamide with *Persea americana* extracts (200 and 300) mg/kg groups in all cycles , in addition to significantly decreased (P<0.05) as likened with cyclophosphamide treated assembly in all cycles except in cycle one.

In current study, the hours of diestrus phase of cyclophosphamide and *Persea americana* extracts at doses (200 and 300) mg/kg treated groups displayed a significantly reduced (P<0.05) as likened with cyclophosphamide treated group and *Persea americana* extract (100mg/kg) and cyclophosphamide treated group in all cycles, Furthermore, there was no significant difference (P<0.05) among cyclophosphamide with *Persea americana* extract (200 mg/kg) treated group , cyclophosphamide with *Persea americana* extract (300 mg/kg) treated group and control group in hours length of diestrus in all cycles.

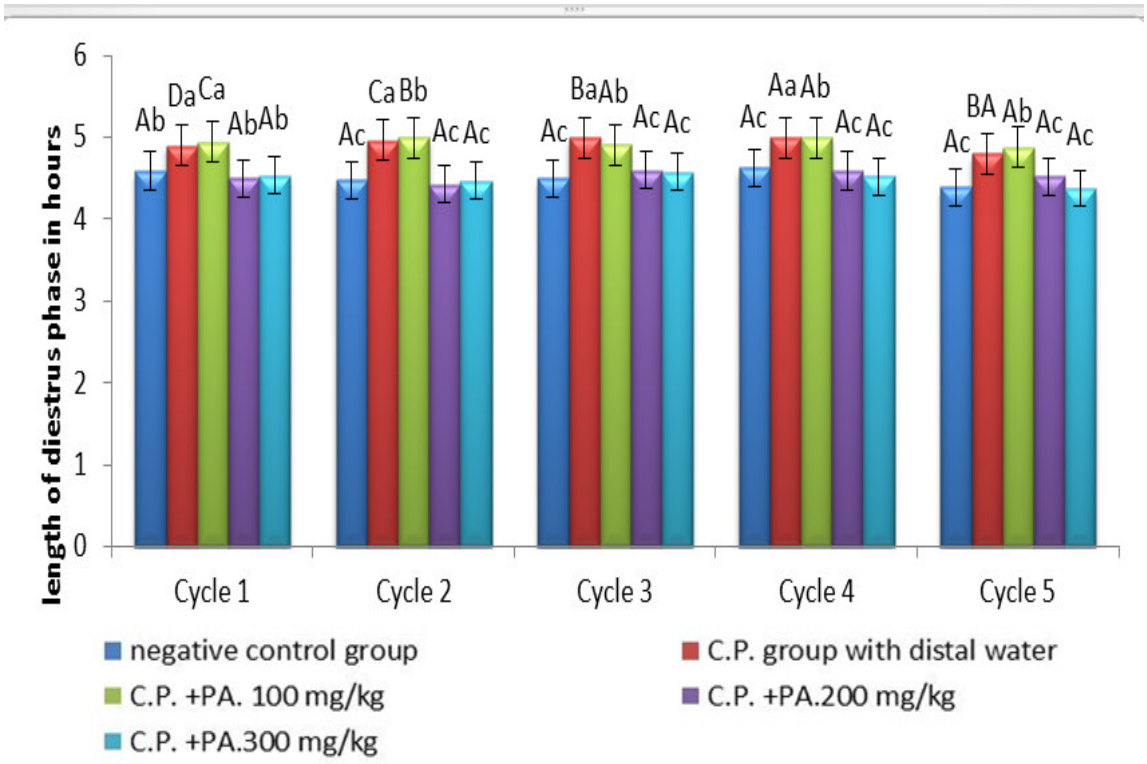


Figure (7): Effect of cyclophosphamide 2mg/kg, cyclophosphamide and *Persea americana* extract (100, 200 and 300)mg/kg and distilled water on di estrus phase of five estrous cycles in hours. *Various capital letters referred to significantly variance (P< 0.05) between groups.*Various small letters referred to significantly variance (P< 0.05) within groups. *C.P.; Cyclophosphamide **P.A*: *Persea americana*

The days of estrous cycles was Illustrated in figure (8), the oral administration of distilled water with cyclophosphamide and cyclophosphamide with *Persea americana* extract 100mg/kg to rats caused a significant surge (P<0.05) in days of estrous cycles three, four and five as likened with baseline group and other collections, while in cyclophosphamide and *Persea americana* extract (200 and300) mg/kg treated groups there were no important variance (P<0.05) in days of estrous cycles as likened with baseline group, furthermore to significantly reduced (P<0.05) as likened with cyclophosphamide treated group and cyclophosphamide with *Persea americana* extract 100mg/kg treated group in estrous cycles (three, four and five).

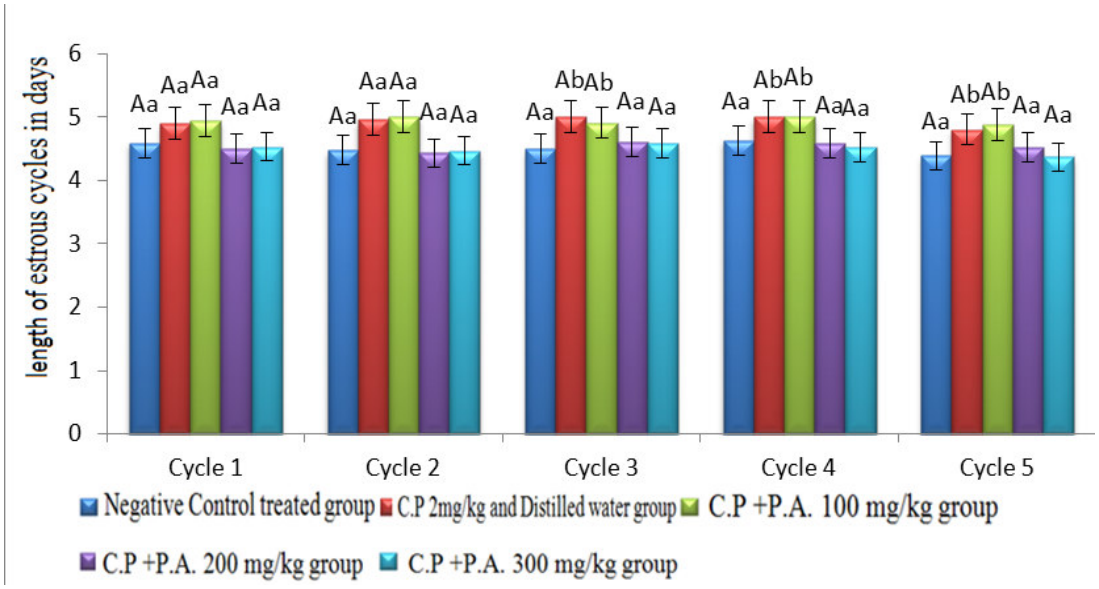


Figure (8): Effect of cyclophosphamide 2mg/kg, cyclophosphamide and *Persea americana* extract (100, 200 and 300)mg/kg and distilled water on estrous cycles in days. *Various capital letters referred to significantly

variance (P< 0.05) between groups. *Various small letters referred to significantly variance (P< 0.05) within groups. *C.P.; Cyclophosphamide *P.A*: *Persea Americana*

4. Discussion

Previous starting this study the estrous cycles was identified to confirm the fertility and regularity the estrous cycle of all female rats, and the outcomes of photomicrographs in all phases decided with results testified by Lohmiller and Swing [8,9], they declared the categorization of every stage from stained swaps achieved from the epithelial of vagina, containing the following: through the cycle of estrous the cornified enucleated acidophilic cells are perceived, while in metestrus, leukocytes are also detected and revealed, the diestrus revealed numerous leukocytes whereas in proestrus phase, pre-acidophilic cells and large basophilic, nucleated acidophilic cells (NAC) cell can be observed. The oral administration of distilled water and cyclophosphamide caused abnormality in all estrous phases, which included prolonged the proestrous, metaestrus and diestrus hours, furthermore, reduced the length of estrus hours and augmented the length of estrous cycle days , this result may be regarded to the cyclophosphamide treatment at its therapeutic dose in animals caused reticence of gonads steroidogenesis, elevating in levels of LH and FSH with decrease, progesterone, estrogen serums and granulosa cell and oocyte apoptosis, the higher levels of gonadotropin result in flaw in ovaries and can be produced amenorrhoea, Cyclophosphamide also motivated on hypothalamus- pituitary- axis connotation which panes all of the reproductive variations beside estrous cycles and caused upsurge level of Gonadotropins releasing hormones and estrous cycles abnormalities, furthermore, lead to infertility because it's a strong ovarian toxicity this result agreed with Oktem, *e al*., [10,11]. The estrous cycles measurements, were altered to the normal sequences after administered rat with *Persea americana* extract at doses 200mg/kg and 300mg/kg, this result can be explained by *Persea americana* has high amounts of folic acid which saved the fertility of female and supported reproductive performance, folic acid consumption was correlated with minor dangerous of ovulation abnormality and infertility. Furthermore, it have Vitamin B6 which assisted in successful fertility by dropping in body inflammation,[12, 13] in addition to present of carotenoid which is an important to the females and also vitamin E that is recognized as vitamin of reproductive power and an energetic fertility, in addition to an exceptional foundation of un saturated lipid which is important for hygienic hormones function, the *Persea americana* plant enclosed the calcium and carbohydrates, the Ca assisted to standardize menstrual cycles [14,15]. While the oral administration of *Persea americana* extract at dose100mg/kg with cyclophosphamide did not altered the estrous cycle to normal sequences this result may be referred to fall the positive effect of *Persea americana* at low dose in contrast to the adverse effect of cyclophosphamide on the estrous cycles and reproductive performance, the similar result told by Petrik,*et al*.[16].

5. Conclusion

This study established that extracts at doses (200mg/kg and 300mg/kg) have more defense consequence on fertility than dose (100mg/kg) of extract in contrast to cyclophosphamide adverse effect .

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