# EFFECT OF *VITEX AGNUS-CASTUS* EXTRACT ON SOME PHYSIOLOGICAL PARAMETERS OF MICE (MUS MUSCULUS L.)

**Mustafa abd almajeed Hussein**

## ABSTRACT

***Vitex*** **(known locally as kaf murium) has along history of traditional use in women. The effect of alcoholic extract of *vitex agnus-castus* was examined for its effect on some blood parameters in mice (Mus Musculus). Mice in the treatment groups were injected intraperitoneally with two doses (6 mg and 12 mg / mouse) of *vitex* extract while the control group received distilled water daily for two weeks. Blood parameters (RBC, Hb, PCV) showed a significant decrease with both doses in comparison to the control group (P<0.01), while no significant decrease in (WBC) was found. Biochemical parameters (total serum protein, total serum cholesterol) showed a significant decrease in the treatment groups as compared with the control group (P<0.01). In conclusion, *vitex agnus-castus* alcoholic extract can have a significant adverse effect on blood. Its cholesterol lowering effect worths investigation in the future.**

## INTRODUCTION

*Vitex* or chestberry is one of the verbenaceae family comeback to *(order Lamiales, class Magnoliopsida, and division magnoliophyta)* native to the Mediterranean and central Asia[1]. It can be grown as a large, deciduous, multistemmed shrub with small and narrow leaves, green on top. It produces long spikes of lavender flowers[2]. *Vitex* has been used for hundreds of years to regulate the function of the reproductive organs in women[3]. It is also thought to exhibit a normalizing or balancing effect on hormone production, and to increase luteinizing hormone (LH) levels without affecting follicle stimulating hormone (FSH) in women[4]. Phytomedicinal preparations containing *vitex* extract have been used in traditional medicine to treat premenstrual tension[5], and used to prevent uterine fibroids, menopausal symptoms and menorrhagia. However, its mechanism of action has not been established[6,7]. Characteristic constituents of the *vitex agnus-castus* leaf include essential oils, glycosides, flavonoids and also labdan diterpenoids, rolundifuran, vitexilactone which have high binding affinity to dopamine receptors[8,9]. In addition flavonoids may have antiviral and antioxidant effect and positive effect on the heart blood vessels[10].

*The aim of this study* is to investigate the potential effect of *vitex* alcoholic extract on blood parameters and also on total serum protein and cholesterol which up to our knowledge have not been studied before in mice.

## Materials and Methods

### *Plant material*

*Vitex* leaves were collected from the garden of the College of Education, University of Basrah and authenticated at the Department of Biology, Colleges of Science and Education. The fresh material (200 gm) was dried in air under shade, and crushed to get a fine powder, then it was boiled in a soxhalate apparatus with 1.5 liter of 96% ethanol for eight hours. After that the crude extract was obtained after removal of the solvent through vacuum pressure evaporation and the extract was stored in a refrigerator for biological studies (a yield of 257 mg of the extract powder was obtained).

### *Animals*

Twenty four adults female mice weighting (22-25 gm) were obtained from the animal house of the Department of Biology, College of Education. They were kept in plastic cages with free access to water and food, with a 12 h dark and 12 h light cycle.

### *Experimental groups*

According to the LD50 of *vitex agnus - castus* extract which is 1650 mg / kg [11], doses used in this experiment were calculated to be (6 mg and 12 mg / mouse). The extract was diluted in distilled water and administerted intraperitoneally (0.1ml) to two groups (eight mice per group) in doses of (6 mg and 12 mg / mouse) daily for 14 days (a preliminary study had shown that two but not one week was enough to obtain a statistically significant

**Mustafa Abd Almajeed Hussein M.Sc., Assistant Lecturer, Department of Physiology , College of Medicine , University of Basrah, Iraq.**

effect). Another group served as the control and received (0.1ml) of distilled water intraperitoneally daily for two weeks. After the last injection, animals were killed and blood samples were collected from the heart and stored in two types of tubes (one with anticoagulant and another without anticoagulant) until assayed.

***Hematological tests***

Blood sample (1-ml) was obtained after 14 days of injection from each of the animals. This blood was used for the determination of blood parameters under investigation: packed cell volume (PCV), hemoglobin percent (Hb) and red blood cell (RBC), and white blood cell (WBC) counts. Packed cell volume was measured by microhematocrit centrifuge spinning for (5 min at 12000 rpm) before reading with the hematocrit reader. Red blood cell and white blood cell counts were estimated by using the hemocytometer methods, and hemoglobin percent was measured by Sahli method.

***Biochemical tests***

A serum sample was obtained from the blood after centrifugation for 5 min at 3500 rpm. The serum was used for measurement of total serum protein and total serum cholesterol using the kit Biocon diagnostic (Germany) and the results were measured by a spectrophotometer.

***Statistical analysis***

Data were analyzed by using SPSS version 9. The results were expressed as the mean ± SD. The significance of the mean difference between the control group and each of treatment groups was determined by revised least significant difference (RLSD).

## RESULTS

The *vitex* extract resulted in a significant decrease in (RBC), (PCV), (Hb), total serum protein, and total serum cholesterol in comparison to the control ($P < 0.01$). No significant decrease in (WBC) in the two treatment groups was found (Table-1). *Vitex* extract (12mg/mouse) produced 24.7% reduction in RBC count; 24.3% reduction in PCV and lowered Hb by 26.8% when compared with control (Table-1).

***Table 1. The effect of vitex alcoholic extracts (6 and 12 mg / mouse) on blood parameters.***

| Treatment (mg/mouse) | RBC×$10^6$ $mm^3$ | WBC (No. / $mm^3$) | PCV % | Hb gm% |
|---|---|---|---|---|
| **Control** | 7.129 ± 1.31 | 4412 ± 1.90 | 37 ± 0.47 | 12.7 ± 1.89 |
| **Extract (6 mg)** | 5.950 ± 0.18 | 4560 ± 1.44 | 31 ± 0.19* (16.2%) | 11.3 ± 0.7 |
| **Extract (12 mg)** | 5.730 ± 0.07* (24.7%) | 4152 ± 2.17 | 28 ± 3.3* (24.3%) | 9.3 ± 0.14* (26.8%) |

A significant decrease in total serum protein after the use of both 6mg dose (*a decrease by 23.6%)* and 12mg dose *(a decrease by 32.7%)* was found (Table-2), while total serum cholesterol was reduced by 61% and 67.3% after using 6 mg and 12 mg doses respectively when compared with control group.

***Table 2. The effect of vitex alcoholic extracts (6 and 12 mg/mouse) on some biochemical tests.***

| Treatment (mg / mice) | Total S. protein gm/dl (% reduction) | Total S. cholesterol mg / dl (% reduction) |
|---|---|---|
| **Control** | 5.5 ± 4.1 | 185 ± 4.3 |
| **Extract (6 mg)** | 4.2 ± 2.4* (23.6%) | 72.2 ± 3.9* (61%) |
| **Extract (12 mg)** | 3.7 ± 3.16* (32.7%) | 60.5 ± 1.24* (67.3%) |

***Significant decrease ($P < 0.01$) in (RLSD) tests.**

## DISCUSSION

The results of this study showed that treatment for 14 days with ethanol extract of *vitex agnus-castus* resulted in a decrease in red blood cell count, packed cell volume, hemoglobin, total serum protein, and total serum cholesterol in female mice (Mus Musculus), while no significant decrease occurred in white blood cells compared with the control group. The significant decrease in blood parameters maybe due to the presence of flavonoids in this plant. It has been shown that the flavonoids can decrease the number of red blood cells and packed cell volume and hemoglobin[12,13]. The essential oils of this plant can also affect blood parameters by inhibition of synthesis of red blood cells[13]. Essential oils and flavonoids were also shown in another study to affect blood parameters resulting in decreased production of its elements[15]. On the other hand, flavonoids can decrease total serum protein and cholesterol. Anderson[16] pointed out that blood cholesterol and protein levels were reduced by using a plant extract containing flavonoids. James and Anderson[17] found that flavonoids can dramatically lower blood levels of cholesterol and reduce the rate of formation of oxidized (LDL). The exact mechanism of flavonoid mediated lowering effect on cholesterol and proteins[16,17] are not yet established. Finally, it can be concluded that *vitex agnus castus* extract can have a significant adverse effect on blood parameters and can lower total serum protein and cholesterol.

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