Effect of Different Levels of (*V. Agnus-Castus*) on the Immunological & Histopathological Changes in Hepatic Rats

Safia Mohammed Bahshwan[1]*

[1]Department of Biology, AL-Baha University, Al-Makhwah, Saudi Arabia.

ABSTRACT

The patient number pursuing alternative and herbal treatments is enormously rising. Herbal medicines are the therapeutic experiences mixture of practicing generation's physicians of indigenous medicine organizations for over hundreds of years. Herbal medicines are now in countless mandates in developing nations for primary healthcare not for the reason that they are reasonably priced and for improved cultural adequacy, enhanced compatibility with the human body. Hence, the study objective is to comprehend the consequence of diverse levels of (Vitex Agnus-Castus) plant on the Histopathological and Immunological alterations in rats injected with carbon tetrachloride (Ccl4). The experiment was carried out in an animal house. All rats were provided for a week on a basal diet before starting the trial, then separated into 2 primary groups; the first group (n= 6 rats) was provided only as a negative control on the basal diet (C –ve) normal rats for 28 days. The rats of the second leading group (n= 24 rats) were injected with carbon tetrachloride (Ccl4). The second leading group is divided into four sub-groups, including three groups fed with different concentrations of Vitex Agnus (5%, 10%, and 15%) and one group control positive infected with the disease did not provide on the experimental diet. The outcomes showed serum IgG insignificant changes between 15%, 10%, and 5% Vitex Agnus-Castus compared with negative control. In contrast, serum GPX showed substantial modifications between 10% and 15% *Vitex Agnus-Castus* than the negative control. In this study *Vitex Agnus* is used to improve the immune system.

Key Words: *V.Agnus, Castus, Immunological changes , Hepatic rats*

eIJPPR 2022; 12(1):38-43

HOW TO CITE THIS ARTICLE: Bahshwan SM. Effect of Different Levels of (*V. Agnus-Castus*) on the Immunological & Histopathological Changes in Hepatic Rats. Int J Pharm Phytopharmacol Res. 2022;12(1):38-43. https://doi.org/10.51847/eWbaaSHAXa

INTRODUCTION

Immunity is the body's capacity to distinguish and eradicate foreign bodies. Henceforth, the immune system is the body's natural defense system against foreign materials penetrating the skin or mucous membranes. Consequently, the hormonal and metabolic changes in the immune system's response that accompany stress, however, suppresses its disease-fighting activity. The immune system will force to work without adequate nutrient support if malnutrition accompanies stress, further impairing its activity. Impaired immunity raises the risk of disease, impairs nutrition, and poor nutrition impairs immunity [1]. The complicated and notable collaborative cells network and its products consist of the immune system. The system exhibits two distinctive traits: Delicate precision and 'Memory.' The specificity is described as an immune cells subcategory that identifies and retorts to the myriad external stimulation a person could experience in a lifetime. In contrast, memory outlined the immune system proficiently, mounting a considerable dynamic and operative reaction the second time a specific excitation was received. The immune system control itself by utilizing cells known as helper suppressor cells and solvable products. It is also in close contact with unique structures inside the body (e.g., the neuroendocrine system) and controlled thru those systems [2].

Plants have continuously played a substantial function in preserving health and humanizing the quality of human life; numerous western medications owe their origin to plant extracts. Therapeutic determinations of native herbs were also developed by several American Indians. Common illnesses or disorders are regularly disbursed in today's list for Self–prescribed herbal preparations. Whereas it is remarkable to note that these herbs are largely

Corresponding author: Safia Mohammed Bahshwan
Address: Department of Biology, AL-Baha University, Al-Makhwah, Saudi Arabia.
E-mail: ✉ sbahashwan@bu.edu.sa
Received: 10 November 2021; **Revised:** 20 February 2022; **Accepted:** 22 February 2022

disbursed in trivial amounts and encompass comparable health-promoting phytochemicals, as do fruits as well as vegetables. There is a chemical and botanical resemblance between countless herbal seasonings and conventional vegetables in the human diet [3]. *Vitex Agnus-Castus* herb is considered one of the famous herbs in the Arab world, used in medicine and alternative because it contains compounds, which made it occupy a distinguished position among the herbs with broad therapeutic and immunological properties. Al-Jalajil, the herb of the city, and the chastity tree, [4].

Aim of investigation

This research aims to understand the upshot of diverse levels of (the Vitex Agnus-Castus) plant on the Histopathological and Immunological changes in rats injected with carbon tetrachloride (Ccl_4).

MATERIALS AND METHODS

Materials

Plants

Taxonomy and Nomenclature of Vitex agnus-castus

Domain: Eukaryota, Kingdom: Plantae, Phylum: Spermatophyta, Subphylum: Angiospermae, Class: Dicotyledonae, Order: Lamiales, Family: Lamiaceae, Genus: Vitex, Species: Vitex agnus-castus.

The plant was designated to research its effects on liver diseases. The local market sold the plants as dried material.

Diets

Basal diet

According to Reeves *et al.* (1993), the basal diet was prepared and entailed protein 20% (casein), sucrose 10%, corn oil 4.7%, choline chloride 2%, vitamin mixture 1%, 3.5% salt mixture and 5% fiber (cellulose). the remainder was corn starch [5].

Carbon tetrachloride (Ccl_4)

Carbon tetrachloride was acquired from El-Gomhoryia Company for Chemical Industries, Cairo, Egypt, as a 10% liquid solution. It was dole out in white plastic bottles, which encompassed 1L as a noxious chemical material for liver poisoning, as stated in Passmore and Eastwood (1986) [6]. Simultaneously, it is mixed with paraffin oil obtained from the pharmacy for dilution during the induction.

Rats

Thirty mature male albino rats of Sprague Dawley strain weighing 150-160 grams b. wt. at the age of 14-16 weeks. Strict hygienic measures were allocated to the animals with metallic stainless covers in plastic cages. Before the experiment commencement for adaptation, the rats were provided with the basal diet for seven days. To avoid food loss and contagion, nutritious foods were offered to rats in distinctive non-scattering feeding cups, and water was delivered *ad libitum* via a narrow mouth bottle with a metallic tube firmly static at its mouth via a piece of rubber tube, as noted before all animals were subjected to a twelve hours dark and twelve hours light schedule and kept for seven days before the experiment commencement for acclimatization.

Methods

Plant Preparation

Dry plant leaves of Vitex *(Vitex Agnus- Castus)* were purchased from the local market of Al-Baha city, Saudi Arabia. All plant materials were pulverized in a mixer to provide ash and were retained in shadowy stoppered glass bottles in a dry location and cooled until use. Plants and herbs are best reserved in a cool, dry, and dark location to decrease their contents oxidation stated by Russo (2001) [7].

Induction of liver intoxication in rats

Thirty male albino rats were treated with carbon tetrachloride (Ccl_4) subcutaneous injection in 50% V/V paraffin oil (2ml / kg b. wt.) two times a week for two weeks to instigate the chronic liver injury according to the process defined by Jayasekhar *et al*., (1997) [8].

Blood samples were obtained by the retro-orbital method after the injection of Ccl_4 to make sure the liver injury incidence and to evaluate liver function.

Grouping and feeding of rats

The study used thirty (30) Sprague Dawley white male albino rats weighing about 150 ± l0g. The rats were separated into 4 groups, each of 6 rats. The groups of rats were as follows:

- Group (1): Control negative group, in which the normal rats fed on a basal diet (control"-").
- Group (2): Control positive group, rats injected with carbon tetrachloride *(CCl_4)* fed on a basal diet (control"+")
- Group (3): received basal diet plus 5% Vitex Agnus
- Group (4): received basal diet plus 10% Vitex Agnus
- Group (5): received basal diet plus 15% Vitex Agnus

Blood sampling

During the final 28 days of the experimentation, the rats were sacrificed by ether anesthesia. The sample of blood was acquired through the retro-orbital technique in a clean, dry centrifuge tube. The samples were kept at room temperature for 20 minutes to clot and then centrifuged at 1500 RPM for 15 minutes. The

biochemical analysis was done after the serum samples were composed by a dry clean syringe, decanted in Wasserman tubes, and then frozen in a refrigerator at -10ºC. Rats were that opened, kidneys, heart, spleen, lungs, and liver removed and washed in saline solution

Immunological analysis

Lymphocytes (CD^{+8} and CD^{+4}) assessments by using mouse monoclonal antibody chromatographic assay according to the described method Boyum (1968) & Burrels and Wells (1977) [9, 10].

Determination of iron indices

- *Determination of serum ferritin*
  Serum ferritin was measured according to Cook *et al*. (1974) [11]. Ferritin, an iron-strong protein, is produced in most body cells and is delectable in the serum of ordinary persons.
- *Determination of Hemoglobin (Hb)*
  Determination of Hemoglobin was according to *Cook et al. (1974).*
- *Hematocrit Determination (PCV)*
  Packed cell volume (PCV) Hematocrit was measured according to Cook *et al*. (1974).

Histopathological examination

According to Carleton, 1978, at the end of the experiment, specimens from the liver were composed right after animals were sacrificed and then fixed in 10% neutral formalin, dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin wax 4-6 thick segments were organized and stained with hematoxylin and eosin.

Statistical analysis

The attained data were evaluated with SPSS software, SAS Institute, Cary, NC). Different treatments effects were investigated by one-way ANOVA (Analysis of variance) test by Duncan's multiple range test, and $p<0.05$ was utilized to designate importance between diverse groups. The following formulas were used [12].

RESULTS AND DISCUSSION

This investigation aims to understand the impact of dissimilar levels of (the Vitex Agnus-Castus) plant on the Immunological and Histopathological variations in rats injected with carbon tetrachloride (Ccl4).

Immunological results

The feeding distinct concentration influence of *Vitex Agnus-Castus* on immunity indices (serum IgG, IgM, and total immunoglobulin) in regular and hepatitis rats after four weeks of feeding were represented in **Table 1**.

- *Serum IgG* value in the regular rat group was (2850.5±350.10) U/ml. Whereas hepatitis rats groups served on basal and enhanced diets with altered levels of *Vitex Agnus-Castus* (positive control, 15%, 10%, and 5% *Vitex Agnus-Castus*) revealed serum IgG values (1500.7±300.51, 2540.9±210.50, 2350.60± 100.30, and 1800.6±250.2) U/ml, respectively. The outcomes established insignificant modifications amongst 15%, 10%, and 5% *Vitex Agnus-Castus* than the negative control.
- *Serum IgM value* in the regular rat group was (380.92±15.12) U/ml. Even though in hepatitis rats, groups provided on basal and augmented diets with diverse levels of *Vitex Agnus-Castus* were (190.55±10.24, 295.40±10.22, 260.40±45.30, and 210.60±15.50) U/ml, for (positive control, 15%, 10%, and 5% *Vitex Agnus-Castus*), respectively. The outcomes affirmed substantial transformations concerning 5%, 10%, and 15% *Vitex Agnus-Castus* related to the negative control.
- *Total immunoglobulin value* in the ordinary rat group was (3650.25±200.5) U/ml. Whereas in hepatitis rats, groups nourished on basal and augmented diets using numerous levels of *Vitex Agnus-Castus* were (2232.78±65.2, 3000.45±65.45, 2700.50±20.50, and 2530.15±60.4) U/ml, for (positive control, 15%, 10%, and 5% *Vitex Agnus-Castus*), respectively. The outcomes established the substantial changes amongst 10%, and 15% *Vitex Agnus-Castus* paralleled the negative control.

Table 1. Effect of different levels of *(V. Agnus-Castus)* on immunity indices

Immunity Indices	Control (+)	Control (-)	15% *Vitex Agnus*	10% *Vitex Agnus*	5% *Vitex Agnus*
IgG (U/ml)	1500.7±300.51^{d}	2850.5±350.1^{a}	2540.9±210.5^{b}	2350.6±100.30^{b}	1800.6±250.2^{c}
IgM (U/ml)	190.55±10.24^{d}	380.92±15.12^{a}	295.40±10.22^{b}	260.40±45.30^{c}	210.60±15.50^{d}
Total Immunoglobulin (U/ml)	2232.78±65.2^{d}	3650.2±200.5^{C}	3000.45±65.4^{a}	2700.50±20.50^{b}	2530.15±60.4^{C}

Values denote arithmetic means ± Standard error of the mean. One-way ANOVA test, whereas those with comparable letters are insignificant.

The feeding different concentration impact of *Vitex Agnus-Castus* on serum detoxifying enzyme indices (serum GSH and serum GPX) in ordinary and hepatitis rats after four weeks of feeding were indicated in **Table 2**.

- *Serum GSH* value in the ordinary rat group was (2.65± 0.03) U/ml. Whereas hepatitis rats groups served on basal and improved diets using diverse levels of *Vitex Agnus-Castus* (positive control, 15%, 10%, and 5% *Vitex Agnus-Castus*) indicated serum GSH values (2.15±0.51, 3.25±0.03, 2.90±0.02, and 2.55±0.03) U/ml, respectively. The outcomes revealed momentous modifications amongst 10% and 15% *Vitex Agnus-Castus* related to the negative control.

- Serum GPX value in the ordinary rat group was (55.90±2.10) U/ml. Whereas in hepatitis rats, groups provided on basal and augmented diets using dissimilar levels of *Vitex Agnus-Castus* were (48.15±2.20, 75.33±9.35, 68.44±6.30, and 55.35±2.40) U/ml, for (positive control, 15%, 10%, and 5% *Vitex Agnus-Castus*), respectively. The outcomes presented substantial variances amid 10% and 15% *Vitex Agnus-Castus* than the negative control.

Table 2. Effect of different levels of *(V. Agnus-Castus)* on detoxifying enzyme indices in rats injected with carbon tetrachloride (Ccl_4)

Serum Detoxifying Enzyme Indices	Control (+)	Control (-)	15% *Vitex Agnus*	10% *Vitex Agnus*	5% *Vitex Agnus*
GSH (U/ml)	2.15± 0.5 ^{b}	2.65 ±0.03 ^{c}	3.25± 0.03^{a}	2.90 ± 0.02^{b}	2.55 ±.03^{c}
GPX (U/ml)	48.15± 2.20 ^{c}	55.90 ± 2.10 ^{c}	75.33 ± 9.35^{a}	68.44 ±6.30^{b}	55.35± 2.40^{c}

GSH (Glutathione), GPX (Glutathione Peroxidase, Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant

Table 3 indicated the different feeding concentrations impact of *Vitex Agnus-Castus* on serum ferritin, hematocrit, and hemoglobin values in ordinary and hepatitis rats after four weeks of feeding.

- *Serum Ferritin* value inside the regular rat group was (48.2 ± 3.1) g/L. Whereas the groups of rats with hepatitis provided on basal and augmented diets using diverse levels of *Vitex Agnus-Castus* (positive control, 5%, 10%, and 15% *Vitex Agnus-Castus*) indicated serum ferritin values (36.3±2.25, 44.9±2.50, 38.25±0.03, and 36.9±4.45) pg/L, respectively. The outcomes indicated substantial changes between all groups of *Vitex Agnus-Castus* when associated with negative control.

- *Hemoglobin* value in the normal rat group was (11.92±0.12) g/L. Whereas in hepatitis rats, groups provided on basal and enhanced diets using diverse levels of *Vitex Agnus-Castus* were (8.20±0.11, 10.95±0.2, 10.66±0.25, and 9.15±0.33) g/L for (positive control, 15%, 10%, and 5% *Vitex Agnus-Castus*), respectively. The outcomes demonstrated a substantial dissimilarity concerning all groups of *Vitex Agnus-Castus* when equated with negative control.

The hematocrit value in the ordinary rat group was (37.25±2.5) %. Whereas in hepatitis rats, groups served on basal and augmented diets using diverse levels of *Vitex Agnus-Castus* were (30.20±2.2, 35.20±4.15, 34.6±2.2, and 31.65±5.4) % for (positive control, 5%, 10%, and 15% *Vitex Agnus-Castus*), respectively. The outcomes established substantial alterations amongst 5%, 10%, and 15% *Vitex Agnus-Castus* related to the negative control. [13], indicated to have an effect on chaste tree fruit (CTF) oral administration extract on development overall performance, serum biochemical parameters, hematological indices, and the increased manifestation and appetite-associated genes in goldfish (Carassius auratus), which corresponds with the prevailing results. The group provided the CTF extract usage at 1.5%, indicating the maximum RBC levels, serum albumin, hematocrit, and hemoglobin ($P < 0.05$). Simultaneously, insignificant modifications were detected for mean hemoglobin, mean corpuscular volume, along with mean corpuscular hemoglobin concentration surrounding entire groups ($P > 0.05$). The aspartate transaminase stages and lactate dehydrogenase have been reduced with the aid of augmenting the CTF extract supplementation tiers, and the lowest values were acquired in a 1.5% CTF extract weight loss program ($P < 0.05$).

Table 3. Effect of different levels of *(V. Agnus-Castus)* on iron indices of rats

Iron Indices	Control (+)	Control (-)	15% *Vitex Agnus*	10% *Vitex Agnus*	5% *Vitex Agnus*
Serum ferritin (g/L)	36.3±2.25^{c}	48.2 ± 3.1^{a}	44.9± 2.50^{b}	38.25± 0.03^{c}	36.9± 4/5°
Hemoglobin (g/L)	8.20 ± 0.1 ^{d}	11.92 ± 0.12^{a}	10.95 ± 0.2^{a}	10.66 ±0.25^{b}	9.15± 0.33^{c}
Hematocrit (%)	30.20 ±3.3^{C}	37.25 ±2.5^{a}	35.20 ± 4.15^{b}	34.60± 2.2^{b}	31.65 ± 5.4^{C}

Values denote arithmetic means ± Standard error of the mean. One-way ANOVA test, while those with similar letters are insignificant

Histopathological results

The histopathological results are considered in **Figures 1-4**.

???

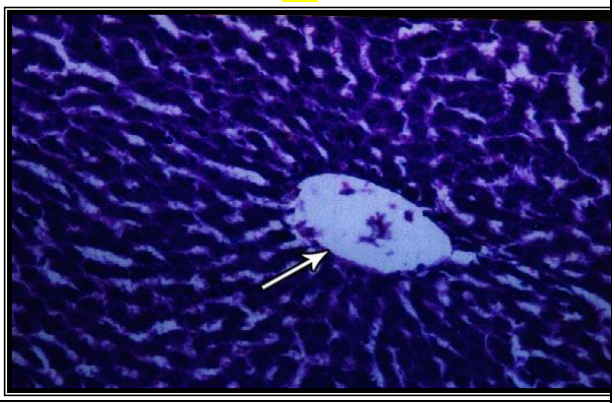

Figure 1. Liver of control (-), untreated rat showing the normal histology of hepatic lobule. (H and E x 200)

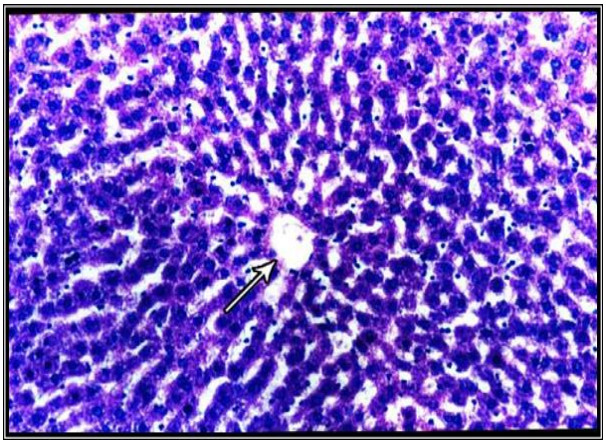

Figure 2. Liver of rat affected with hepatitis and treated with 15% Vitex Agnus showing Simusoidal leucocytosis. (H and E x 200)

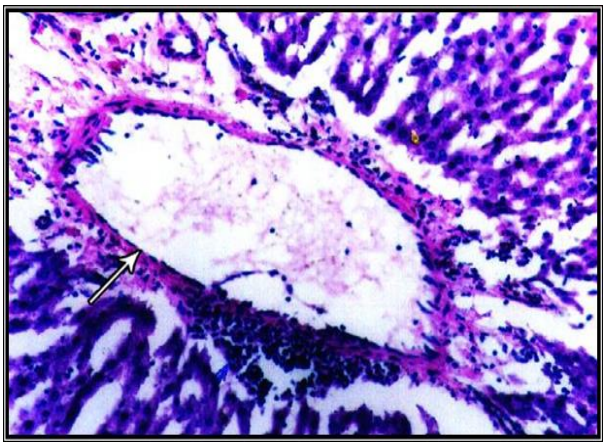

Figure 3. Liver of rat affected with hepatitis and treated with 10% Vitex Agnus showing kupffer cells activation. (H and E x 200)

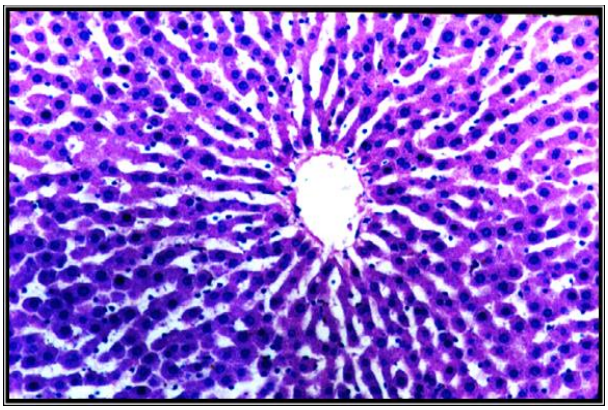

Figure 4. The liver of rats affected with hepatitis and treated with 5% Vitex Agnus indicated no histopathological changes were detected in the rat's liver from groups (H and Ex200)

Recommendations

1. This study recommended using *Vitex Agnus* to improve the immune system.
2. Use different levels of *Vitex Agnus*, especially that of 5%.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

REFERENCES

[1] Turvey SE, Broide DH. Innate immunity. J Allergy Clin Immunol. 2010;125(2):S24-32. doi:10.1016/j.jaci.2009.07.016.

[2] Liu S, Wang S, Lu S. DNA immunization as a technology platform for monoclonal antibody induction. Emerg Microbes Infect. 2016;5(4):e33. doi:10.1038/emi.2016.27.

[3] Boullata JI, Nace AM. Safety issues with herbal medicine. Pharmacotherapy. 2000;20(3):257-69.

[4] Hajdú Z, Hohmann J, Forgo P, Martinek T, Dervarics M, Zupkó I, et al. Diterpenoids and flavonoids from the fruits of Vitex agnus-castus and antioxidant activity of the fruit extracts and their constituents. Phytother Res. 2007;21(4):391-4. doi:10.1002/ptr.2021.

[5] Reeves PG, Nielson FH, Fahmy GC. Reports of the American Institute of Nutrition, adhoc wiling committee on reformulation of the AIN 93. Rodent Diet J Nutri. 1993;123:1939-51.

[6] Passmore R, Eastwood MA. Human Nutrition and Dietetics. Eight editions. Longman Group UK LTD. Churchill Livingstone. 1986.

[7] Russo E. Handbook of Psychotropic Herbs a Scientific Analysis of Herbal Remedies for Psychiatric Conditions. The Haworth Herbal Press, Inc. 2001.

[8] Jayasekhar P, Mohanan PV, Rathinam K. Hepatoprotective activity of ethyl acetate extract of Acacia catechu. Indian J Pharmacol. 1997;29(6):426-8.

[9] Boyum A. Isolation of mononuclear cells & granulocytes from human blood. Scand J Clin Lab Invest. 1968;21:77-89.

[10] Burrells C, Wells PW. In vitro stimulation of ovine lymphocytes by various mitogens. Res Vet Sci. 1977;23(1):84-6.

[11] Cook JA, Kliewer WM, Lider LA. General Viticulture. 4th Edition, University of California Press, Berkley; 1974. 740 p.

[12] Snedecor GW, Cochran WG. Statistical Methods. 6th Ed. Iowa State University Press, Ames, Lowa. USA. 1967.

[13] Rashmeei M, Shekarabi SP, Mehrgan MS, Paknejad H. Assessment of dietary chaste tree (Vitex agnus-castus) fruit extract on growth performance, hemato-biochemical parameters, and mRNA levels of growth and appetite-related genes in goldfish (Carassius auratus). Aquac Fish. 2022;7(3):296-303.