Effect of bee venom on some blood and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug

IOP Conf. Series: Journal of Physics: Conf. Series **1234** (2019) 012066 doi:10.1088/1742-6596/1234/1/012066

Effect of bee venom on some blood and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug

Zainab I. Mohammed [1], Ahmed J.Hassan [2]

[1] Department of Physiology and Pharmacology, College of veterinary medicine, University of AL-Qadisiyah, Iraq.

[2] Department of Biology, College of Education, University of AL-Qadisiyah, Iraq.

Email: zainab.mohammed@qu.edu.iq

Abstract: Background Bee venom(BV) has been used to treat and reduce chronic inflammatory diseases such as rheumatoid arthritis(RA),pain , skin diseases and to treat cancerous tumors. The aim of this study was to investigate the effects of bee venom on some blood and biochemical parameters in male albino rats with Arthritis and compare it with prednisolone drug.

Materials & methods: (42) mature male albino rats (135-150) gm, divided into (7) groups, (6) male rats for each group. The experiment was continued for (14) days and included: the first group (negative control) was injected by (0.1ml/animal)from physiological normal saline (0.9% Nacl),the second group(Arthritis group) was injected by (0.1ml/animal) formaldehyde, the third group (normal group) was injected by bee venom(i.p) (1mg / kg of B.W), the fourth group (normal group) was treated orally with prednisolone (5mg / kg of B.W), the fifth group (Arthritis group) was injected by bee venom(i.p) (1mg / kg of B.W), the sixth group (Arthritis group) was treated orally by prednisolone (5 mg / kg of B.W) and seventh group (Arthritis group) was injected by bee venom (1 mg/ kg of B.W) and treated orally with prednisolone (5mg / kg of B.W).

Results: Our results showed that 2nd group (Arthritis) significantly increased ($p \leq 0.05$) in the number of WBCs and platelets compared with control group, while the 3rd and 4th groups showed a significant decrease in WBCs and PLT compared with 2nd group. The 5th , 6th and 7th groups showed a significant decrease ($P \leq 0.05$) in the number of WBCs and platelets compared with 2nd group. On the other hand, the present study demonstrated a significant decrease ($p \leq 0.05$) in RBCs, hemoglobin (Hb)and hematocrit (HCT) in the 2nd group (Arthritis group) compared with control group, while the 3rd and 4th groups showed a significant increase in RBCs, Hb and HCT compared with 2nd group. The 5th , 6th and 7th groups showed increase ($p \leq 0.05$) in RBCs, Hb and HCT compared with 2nd group. The results showed also, a significant increase in levels of cholesterol, triglycerides , LDL and a significant decrease in HDL level in 2nd group compared with control group, while 3rd,4th, 5th ,6th and 7th groups showed a significant decrease ($P \leq 0.05$) in cholesterol, triglycerides, LDL and a significant increase in HDL level compared with 2nd group.

Conclusion: We conclude from the present study that bee venom attenuates development of Arthritis by improving some Blood and Biochemical parameters.

1. Introduction

Rheumatoid arthritis is one of the most autoimmune disease prevalence in the present time and it is the most common in the female at ratio 3:1[1], it causes many adverse effects in the body systems include lymphatic, hematology, respiratory and muscular system as well as, it's effect on heart and kidneys [2]. RA is a disease condition, occurs in any age especially between 35-50 age [3]. There are many factors that play an important role in appearance of RA such as heat shock protein- 70, smoking, Rheumatoid factor, stress and cytokines especially tumor necrosis factor[4,5]. Many drugs are used to treat RA such as Non- steroidal anti-inflammatory drugs (NSAIDS), Disease modifying anti rheumatic drugs (DMARDS) and corticosteroids such as prednisolone and prednisone [3].

Glucocorticosteroids are used as a single agent or in combination with other anti-rheumatic drugs. In short-term (duration of use <1 year) regimens, treatment is often aimed at controlling symptoms in periods of high disease activity either with unchanged background anti-rheumatic therapy or while awaiting the effects of newly started other disease-modifying drugs[6], but used of these drugs for long period, cause adverse effects in cardio vascular system, Renal failure, an ulcer in stomach and intestine[3].

In the recent time, researchers have been directed towards used of anti-inflammatory from natural sources because the adverse effects of drugs that used to treat arthritis. One of these natural sources, Bee venom that has both anti-inflammatory and anti-microbial effects involving no side effects in animal model [7]. BV composed of melittin which forms the main component of BV, as well as apamin, adolapin, phospholipase A2, Hyalurornidase, biologically active amines such as (Histamine, Epinephrine), also BV contains lipids, carbohydrates and free amino acids [8,9].

This experiment was designed to study the protective effect of bee venom on some blood and biochemical parameters in male albino rats with Arthritis and compare it with prednisolone drug.

2. Materials and methods

2.1 Experiment animals

42 male albino rats (135-150) gm. Were obtained from animal house of vet. med. College, University of AL-Qadisiyah. The animals were placed in plastic cages in an air conditioned room with temperature maintained at 25 ± 2 Cº. the experiment was performed under controlled conditions (temperature, humidity and 12 h light –dark cycle Rats were given sterile food pellets and tap water. All rats were divided randomly in to (7) groups, (6) rats for each one and were treated as below for (14) days:

1. First group(1st) :Which treated as negative control group, it was injected with (0.1 ml/ animal) from physiological normal saline (0.9% Nacl) in the first and third day of the experiment.
2. Second group(2nd): (Arthritis group): which treated as positive control it was injected with (0.1 ml/ animal) of formaldehyde (2%) in the first and third day of the experiment.
3. Third group(3rd): (Bee venom group) it was normal group, injected by Bee venom (i.p) (1 mg/ kg of B.W once daily for 14 days).
4. Four group(4th): (prednisolone group): it was normal group, treated orally with prednisolone (5 mg/ kg of B.W once daily for 14 days.
5. Fifth group(5th): (Bee venom + arthritis) : it was arthritis group, injected by BV (i.p) (1 mg/ kg of B.W once daily for 14 days.

6. Sixth group(6th): (arthritis + prednisolone) : it was arthritis group, treated orally with prednisolone (5mg/ kg of B.W once daily for 14 days).
7. Seventh group(7th): (arthritis + BV+ prednisolone): it was arthritis group, injected by BV (i.p) (1mg/ kg of B.W) and treated orally with prednisolone (5 mg/ kg of B.W one daily for 14 days).

2.2 Chemicals:

Bee venom (BV) was obtained from (Sigma-Aldrich, USA) in packages contain (1gm) of BV powder, prepare at a dose (1mg/ kg of B.W) according to(10).Prednisolone was obtained from market and prepare at a dose (5mg/ kg of B.W) according to[11].

2.3 Induction of arthritis:

Arthritis was induced in rats by administration of (0.1 ml) formaldehyde (2%) in to the right hind paw in the first and third day of the experiment [12].

2.4 Blood collection:

Blood collection was done at 14 days of the experiment via abdominal vein. Blood samples were collected after 14 days placed (2ml) of blood in test tubes with anti-coagulated to hold blood parameters and (3ml) of blood placed in test tubes without anti- coagulated that allowed coagulating for 15 min. Serum was separated from coagulated blood samples by centrifugation at 3000 rpm for 15 min and then kept in the frozen at -20C^{o} until using to estimate lipid profile.

2.5 Estimation of blood parameters:

Blood parameters was measured by Hematology analyzer apparatus which include: WBCs, RBCs, Hb, HCT and platelets (PLT).

2.6 Estimation of lipid profile:

1-cholestrole and HDLC were estimated according to [13].
2.Triglycerides were estimated according to [14].
3.LDL-C was estimated according to [15].

2.7 Statistical analysis:

Grouped date was statistically read by spss program, methods of testing include one way ANOVA for comparisons among groups followed by least significant differences (LSD) test to compare among groups, P values of $P \leq 0.05$ were considered to record statistical significance, all data were expressed as mean ± standard error (SE) (16).

3. Results

3.1 Blood parameters

Our results showed that 2nd group significantly increased ($P \leq 0.05$) in the value of WBCs (14.18 ± 1.42) compared with control group. The 3rd and 4th groups showed a significant decrease in WBCs compared with 2nd group, the 3rd and 4th groups showed non significant changes in WBCs compared with control group. Also the results showed a significant decrease in the value of WBCs in 5th (6.50 ± 0.81), 6th (5.63 ± 0.44) and 7th (3.13 ± 0.67) compared with 2nd group. The 5th group showed non significant increase in WBCs compared with 6th group as in table (1).

The results showed a significant decrease ($P \leq 0.05$) in RBCs value (5.21 ± 0.17), Hemoglobin (Hb) (10.30 ± 0.42) and Hematocrit value (33.12 ± 1.84) in the 2nd group

compared with control group. The 3rd and 4th groups showed a significant increase in RBC, Hb and HCT compared with 2nd group, while these groups showed non significant changes in RBCs, Hb and HCT compared with control group. Also the results showed an increase in RBCs, Hb and HCT values in the 5th, 6th and 7th groups compared with 2nd group, while 5th group showed non significant increase in RBCs, HCT and a significant increase in Hb compared with 6th group as in table(1).

Platelets values showed significantly increased (P≤ 0.05) in the 2nd group (739.75± 38.66) compared with control group, The 3rd and 4th groups showed a significant decrease in PLT value compared with 2nd group, while the 4th group showed non significant changes in PLT value compared with control group. Also the results showed a significant decrease (P ≤ 0.05) in PLT values in the 5th group (390.25± 43.21), 6th group (462.25± 29.36) and 7th group (552.50 ± 8.64) compared with 2nd group, while the 5th group showed non significant decrease in PLT value compared with 6th group as in table(1).

Table 1. Effect of Bee venom and prednisolone on blood parameters in studied groups.

	WBC	**RBC**	**HB**	**HCT**	**PLT**
1st	4.72±0.26 C	7.18±0.37 A	12.56±0.33 AB	37.52±0.43 A	446.75±47.19 Bg
2nd	14.18±1.42 A	5.21±0.17 C	10.30±0.42 C	33.12±1.84 B	739.75±38.66 A
3rd	5.20±0.70 BC	7.06±0.26 AB	12.75±0.18 AB	37.40±0.67 A	372.75±19.24 Cg
4th	5.23±0.31 BC	7.81±0.08 A	13.47±0.57 A	41.12±1.67 A	484.74±90.15 Bg
5th	6.50±0.81 B	5.98±0.48 C	11.52±0.30 B	34.72±1.78 AB	390.25±43.21 Cg
6th	5.63±0.44 BC	5.73±0.36 C	10.55±0.46 C	33.57±2.75 AB	462.25±29.36 BC
7th	3.13±0.67 D	6.26±0.38 B	12.07±0.60 B	37.40±1.55 A	552.50±8.64 B
L.S.D	1.56	0.80	1.05	4.20	112.69

NOTE: Results expressed as mean± S.E
-Different letters refer to significant differentiations between groups at (P≤0.05).
-Similar letters refer to no significant differentiations between groups at (P≤0.05).
-1st group: control group.
-2nd group: first treatment group (Arthritis group).
-3rd group: second treatment group(normal group treated with bee venom at a dose 1mg/kg of B.W).
-4th group: third treatment group(normal group treated with prednisolone at a dose 5 mg/kg of B.W).
-5thgroup:fourthtreatmentgroup(arthritis group treated with BV at a dose 1mg /kg of B.W).
-6th group: fifth treatment group(arthritis group treated with prednisolone at a dose 5 mg/kg of B.W).
-7th group: sixth treatment group((arthritis group treated with bee venom at a dose 1mg/kg of B.W and with prednisolone at a dose 5 mg/kg of B.W).

3.2 Biochemical parameters

Our results showed that 2nd group significantly increased (P ≤ 0.05) in the values of cholesterol (86.085± 1.328), triglycerides (62.445± 0.317) ,LDL (48.435± 0.439) and significantly decreased (P ≤ 0.05) in the value of HDL(28.150± 0.152) compared with control group. The 3rd and 4th groups showed a significant decrease (P ≤ 0.05) in the values of

cholesterol, triglycerides, LDL and a significant increase in HDL level compared with 2nd group.

Also results showed a significant decrease ($P \leq 0.05$) in the values of cholesterol, triglycerides , LDL and a significant increase in value of HDL in the 5th , 6th and 7th groups compared with 2nd group, while the 5th group showed a significant decrease in the levels of cholesterol, triglycerides, LDL and non significant increase in the HDL level compared with 6th group, as in the table (2).

Table 2. Effect of Bee venom and prednisolone on lipid profile in studied groups.

	Cholesterol	**TG**	**HDL**	**LDL**
1st	72.085±0.369 D	42.445±0.317 E	48.150±0.152 A	31.185±0.081 E
2nd	86.085±1.328 A	62.445±0.317 A	28.150±0.152 E	48.435±0.439 A
3rd	69.585±0.413 E	39.655±0.435 F	47.437±0.073 F	29.492±0.491 G
4th	73.585±0.273 D	44.595±0.625 D	49.575±0.054 D	32.135±0.265 E
5th	77.192±0.223 C	51.340±0.323 C	37.385±0.098 C	37.242±0.181 D
6th	79.942±0.610 B	56.090±0.436 B	36.885±0.304 BC	39.992±0.312 B
7th	79.192±0.223 B	54.590±1.275 B	36.635±0.459 B	38.992±0.438 C
L.S.D	1.48	1.50	0.67	0.83

Results expressed as mean± S.E
-Different letters refer to significant differentiations between groups at (P≤0.05).
-Similar letters refer to no significant differentiations between groups at (P≤0.05).
-1st group: control group.
-2nd group: first treatment group (Arthritis group).
-3rd group: second treatment group(normal group treated with bee venom at a dose1mg/kg of B.W).
-4th group: third treatment group(normal group treated with prednisolone at a dose 5mg/kg of B.W).
-5thgroup:fourth treatment group(arthritis group treated with BV at a dose1mg/kg of B.W).
-6th group: fifth treatment group(arthritis group treated with prednisolone at a dose 5 mg/kg of B.W).
-7th group: sixth treatment group(arthritis group treated with bee venom at a dose1mg/kg of B.W and with prednisolone at a dose 5 mg/kg of B.W).

4.Discussion

The object of the present study was to identify the pharmacological effects of bee venom on arthritis

4.1 Blood changes

The results of the present study showed a significant increase in the values of WBCs ($P \leq 0.05$) in the 2nd group compared with control group, the cause of these results belong to inflammation that accompanying with the onset of arthritis induced [17] or these result may be due to the inflammatory processes that occur in the body that lead to produce some inflammatory cytokines which in turn causing increased proliferation of WBCs [18]. Also an increase in the total number of WBCs in the first treatment group may be due to exposure the animals to oxidative stress which causes an increase in the secretion of ACTH from pituitary

gland which in turn causing increased in the production of corticosterone hormone from cortex of adrenal gland, therefore the number of WBCs increase [19].

The significant decrease in the total number of WBCs ($P \leq 0.05$) in the 5th group compared with 2nd group may be due to Immunosuppressive effect of bee venom that cause decrease in the total number of WBCs [20], While the significant decrease in the total number of WBCs ($P \leq 0.05$) in the 6th group may be due to the ability of prednisolone to prevent the infiltration of WBCs to the inflamed tissues[21,22].Also [23] showed that prednisolone and Atorvastatin have anti-inflammatory effect and contribute in the reduction of WBCs count, through preventing recruitment and adhesion of WBCs to the vascular endothelium and the anti-inflammatory effect of prednisolone and Atorvastatin may be due to inhibition of the production of isoprenoids which contribute in the biosynthetic pathway of cholesterol, as well as isoprenoids have effects on G-protein, adhesion molecules and cell proliferation and blocking the production of isoprenoids has many effects on the functional systems which related with inflammation such as WBCs [24,25].

The present study showed that the 7th group demonstrated a significant decrease in the total number of WBCs and this because of the combination effect of BV and prednisolone.

Our present results showed a significant decrease in the values of RBCs, Hemoglobin(Hb) and hematocrit(HCT) in the 2nd group (first treatment group) compared with control group, these due to the disease activity in bone marrow suppression or erythropoiesis may be inactive[26] and these decreased may be due to defect in the function of thyroid gland because of toxins secretion that generate in the body of animal as a result of arthritis induction by formaldehyde, hormones of thyroid gland affect on metabolism processes in the body as well as their in direct effects on production of RBCs from bone marrow[27].

The results showed a significant increase ($P \leq 0.05$) in the values of RBCs, hemoglobin (Hb) and non significant increase in the value of hematocrit(HCT) in the 5th group compared with 2nd group, these results because of BV increases coronary and peripheral circulation and improves circulation of blood in the micro blood vessels, as well as its role in stimulation of building erythrocytes [28]. [29] showed that BV has preventive role in the protection of the body from radiation and contribute in the improvement renewal of RBCs in the rats that exposure to gamma radiation, While the significant increased in the values of RBCs, Hb and HCT($P \leq 0.05$) in the 6th group (fifth treatment group) due to role of prednisolone in the improvement of blood parameters such as RBCs, Hb and HCT, where it works on the regulation of hematopoiesis and stimulation of erythropoiesis in direct form through increasing the production of erythropoietin in kidney[30, 31].The significant increase in the values of RBCs, Hb and HCT($P \leq 0.05$) in the 7th group (sixth treatment group) due to the combination effect of BV and prednisolone.

The platelets results showed that the 2nd group (first treatment group) significantly increased ($P \leq 0.05$) in the PLT value compared with control group, due to present of Rheumatoid arthritis inflammation in the early stage [32], Other studies showed these increasing in the PLT value due to the great effect of cytokines especially IL-6, where it works on the maturation of generated cells of PLT and causes increasing production of PLT [33].

The results showed a significant decrease in the value of PLT ($P \leq 0.05$) in the 5th group compared with 2nd group because of BV has antioxidant effect(29), where the antioxidants act

to reduce the damages that result from oxidative stress[28], oxidative stress occurs as a result of inflammatory cytokines especially IL-6 that causes an increase in the number of PLT and BV works on inhibition the activity of IL-6 and thus less number of PLT[34], While the 6th group demonstrated a significant decrease in the value of PLT ($P \leq 0.05$) compared with 2nd group, these result due to the role of prednisolone to control on the deterioration in the blood parameters as a result of arthritis, as well as prednisolone has anti rheumatoid and antioxidant effects [31].Finally, the significant decrease in the number of PLT ($P \leq 0.05$) in the 7th group due to the combination effect of BV and prednisolone.

4.2 Biochemical changes:

Our results showed a significant increase ($P \leq 0.05$) in the values of cholesterol, triglycerides, LDL and significantly decrease in the value of HDL in the 2nd group compared with control group, these results due to systemic inflammation occurs as a result of elevation C- reactive protein and ESR concentration which consider independent risk factor and play an important role in atherosclerosis, as well as inflammatory mediators such as cytokines, chemokines and immune cells cause many metabolic effects in arthritis and these inflammatory mediators play an important role in atherosclerosis and dyslipidemia such as increase LDL, cholesterol, triglycerides levels and decrease HDL level[35,36,37].[38] showed that increasing in the level of cholesterol and TG due to injection the animals with formaldehyde which causing release of toxins in their bodies, these toxins affect on the function of thyroid gland and cause hypothyroidism, reduction the concentration of T3 and T4 hormones affect on metabolism of cholesterol in the body and increased its concentration in the blood. Also reduction the concentration of T3 and T4 hormones adversely affect on lipoprotein lipase that works on analyze of TG, therefor the concentration of TG increase in the blood [39].

[40], showed that elevation of cholesterol level in the animal blood infected by arthritis related directly with level of LDL and adversely with level of HDL and this explain the significant increase in the level of LDL and significant decrease in the level of HDL.

The results showed that the 5th group (fourth treatment group) significantly decreased ($P \leq 0.05$) in the levels of cholesterol, TG,LDL and significantly increased in the level of HDL because of Bee venom has hypolipidemic activity in the mice induced by diabetes [41] and this hypolipidemic effect of BV attributes to melittin, phospholipase A2 and poly peptide that are together make up more than 62% of BV[42]. There is another mechanism explains reason of dyslipidemia in animals with diabetes, this mechanism is link metabolism of glucose and fatty acid by improving insulin action in fat cells which lead to lower LDL, TG and increased HDL levels [43], also the enzymatic activity of phospholipase A2 in BV plays an important role in reducing the level of cholesterol, TG, LDL and increasing HDL level as well as regulating of lipid profile in the blood [44].

The significant decrease in the levels of cholesterol, TG, LDL and increasing in HDL level ($P \leq 0.05$) in the 6th group (fifth treatment group) compared with 2nd group due to use of prednisolone in low dose causing an elevation in the levels of cholesterol and HDL[45], as well as used prednisolone in high dose has appropriate effect on lipid profile. Also [46], showed that glucocorticoids cause an increasing in the level of HDL and total cholesterol, while [40], showed that the level of cholesterol link inversely with the level of HDL, therefore the reason of this result that the most acceptable is the glucocorticoids more affect on HDL

level [46] and cause increasing the level of HDL, therefore the elevation of HDL causes decreasing level of cholesterol in the blood.

Finally, the significant decrease in the levels of cholesterol, TG, LDL and increasing in HDL level ($P \leq 0.05$) in the 7^{th} group(sixth treatment group) because of the combination effect of BV and prednisolone.

References

[1] Tripathi, K.D.2008. Essentials of Medical Pharmacology. 6th ed. New Delhi, India: Jaypee Brothers Medical Publishers.

[2] Nicola, P.J.; Crowson,C.S.; Maradit-Kremers, H.; Ballman, K.V.; Roger, V.L.& Jacobsen, S.J. et al. 2006. Contribution of congestive heart failure and ischemic heart disease to excess mortality in rheumatoid arthritis. Arthritis Rheum;54:60-7.

[3] Ganesan, R.; Doss, H.M. & Rasool, M.2016. Majoon Ushba, a polyherbal compound ameliorates rheumatoid arthritis via regulating inflammatory and bone remodeling markers in rats, Cytokine, 77 115-126.

[4] Moseley,T.A.; Haudenschild, D.R.; Rose, L. & Reddi, A.H. 2003. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev.14,155–174.

[5] Costanbader, K.H.; Chang, S.C.; De Vivo, I.; Plange, R.& Karlson, E.W.2008. Genetic polymorphisms in PTPN22, PAD1-4 and CTLA-4 and risk for rheumatoid arthritis in two longitudinal cohort studies; evidence of gene-environment interactions with heavy cigarette smoking. Arthritis Res Ther; Epub.

[6] Kirwan JR.1995 The effect of glucocorticoids on joint destruction in rheumatoid arthritis. The Arthritis and Rheumatism Council Low-dose Glucocorticoid Study week randomised controlled trial. Br J Rheumatol Group. N Engl J Med.333:142–6.

[7] Han, S. M.; Lee, K. G. ; Yeo, J. H. ; Kweon, H. Y. ; Woo, S. O. ;.Lee, M. Y.; Baek, H. J. & Park, K.K. 2006. Effect of venom from the Asian honeybee (Apis cerana Fab.) on LPS-induced nitric oxide and tumor necrosis factor-α production in RAW 264.7 cell line. J. Apic. Res. 45:131–136.

[8] Sciani, J.M.; Marques-Porto, R.; Lourenço Junior, A.; Orsi, R. O.; Ferreira Junior, R.S.; Barraviera, B.& Pimenta, D.C. 2010. Identification of a novel melittin isoform from Africanized Apis mellifera venom. Peptides 31, 1473-1479.

[9] Ferreira-Junior, R.S.; Sciani, J.M.; Marques-Porto, R.; Junior, A.L.; Orsi, R.O.; Barraviera, B.& Pimenta, D.C.2010. Africanized honey bee (Apis mellifera) venom profiling: Seasonal variation of melittin and phospholipase A2 levels. Toxicon 56, 355-362.

[10] Lee,J.D.; Kim,S.Y.; Kim,T.W.; Lee,S.H.; Yang,H.; Lee,D. I.& Lee, Y.H.2004. Anti-inflammatory Effect of Bee Venom on Type II Collagen-Induced Arthritis. The American J. of Chinese Medicine, Vol. 32, No. 3, 361–367.

[11] Barua,C.C.; Bodduluru,L.N.; Haloi,P.; Purukayastha,A.; Patowary,P; Hussain, M.& Bora,M. 2017. Anti-arthritic and anti-inflammatory activity of a polyherbal formulation against Freund's complete adjuvant induced arthritis in Wistar rats. Indian J. of Traditional Knowledge. Vol. 16(3), pp. 482-489.

[12] Kore, K.J.; Shete, R.V.& Desai, N.V.2011. Anti-arthritic activity of hydro alcoholic extract of Lawsonia innermis. Int. J. Drug Dev. Res.;3(4): 217-24.

[13] Tietz,N.V.1999."Text book of clinical chemistry ".W.B. Saunders company ,Philadelphia,p:490-491,1000-1025.

[14] Tietz,N.W. 1987 .Fundamentals of clinical chemistry. Saunders company, Philadelphia. pp. 940.

[15] Simon,H. 2006.Cholesterol, Other lipids, and lipoproteins.6th Avenue-Des Moines, Mercy Medical Center,P:1-22.

[16] Leech,N.L.;Barrett, K.C. & Morgan, G.A.2011 .IBM SPSS for intermediatestatistics.4th ed. Taylor and Francis Group. LLC.USA.

[17] Lee, J.Y.; Kang, S.S.; Kim, J.H.; Bae, C.S.& Choi, S.H. 2005. Inhibitory effect of whole bee venom in adjuvant-induced arthritis, In Vivo, P: 801–805.

[18] Hasan,A.&Jassim,H.2011.Effect of treating lactating rats with lead acetate and its interaction with vitamin E or C on neurobehavior, development and some biochemical parameters in their pups. Ir. J.Vet. Sci., 1:45-52.

[19] Kumar,V.;Abbas,A.K.& Fausto, N. 2004.Robbins &Cotran pathologic basis of disease.7th. Philadelphia: Saunders.

[20] Bankhurst, A., Husby, G.& Wiliams, R. 2005. Predominance of t cells in the lymphocytic infiltrates of synovial tissues in rheumatoid arthritis. Arthritis and Rheumatism,Volume 19, Issue 3, pages 555-562.

[21] McCarey, D. W.; Sattar, N. & McInnes, I. B.2005. Do the pleiotropic effects of statins in the vasculature predict a role in inflammatory diseases? Arthritis Res. Ther. 7, 55–61.

[22] Tandon,V.R.; Mahajan, A. & Verma, S.2005. Statins and Rheumatoid arthritis. J Indian Rheumatol Assoc 13,54–59.

[23] Yoon, S.S.& Dillon, C.F.2010..Effects of Statins on Serum Inflammatory Markers : The U. S. National Health and Nutrition Examination Survey 1999 − 2004. J Atheroscler Thromb 17, , 1176–1182.

[24] Stancu, C. & Sima, A.2001. Statins: mechanism of action and effects. J. Cell. Mol. Med. 5,378–387.

[25] Lechleitner, M.2002. Non Lipid Related Effects of Statins. J. Clin. Basic Cardiol. 5, , 205–208.

[26] Harrison, E.D. 2001.Chemical features of rheumatoid arthritis, Textbook of Rheumatology £fh edition W. B. Saunders Philadelphia. 167-169.

[27] Safer,J.D.;Crawford,T.M.&Holcki,M.F.2005.Topical thyroid hormone accelerates wound healing in mice. Endocrinology in press.145:3257-23.

[28] Son, D.J.; Lee, J.W.; Lee, Y.H.; Song, H.S.; Lee, C.K.& Hong, J.T.2007. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol Ther; 115:246–270.

[29] Salman,M.M.; Mohi Eldin,M.M.& Kasem,N.R.A.2015. Physiological effects of Bee Venom and Propolis on irradiated Albino rats. Danish Journal of Agriculture and Animal Sciences.PP11-21.

[30] Von Lindern, M.; Deiner, E. M. & Dolznig H. 2001. Leukemic transformation of normal murine erythroid progenitors: vand c-ErbB act through signaling pathways activated by the EpoR and c-Kit in stress erythropoiesis. Oncogene 20,3651–3664.

[31] Abed El –Gaphar,O.; Abo-youssef,A.M.& Abo-saif,A.A. 2015. Differential Effects of Atorvastatin and Prednisolone on Inflammation, Oxidative Stress and Hematological Biomarkers on Freund's Adjuvant Induced-Arthritis in Rats. Int. J. Pharm. Sci. Rev. Res., 33(2), No. 49, Pages: 235-241

[32] Fit,N.; Chirila,F.; Rapuntean,S.; Nadas,G.; Preoteasa,L.& Cumpanasu,F.2011. Haematological and Biochemical Investigations in Rats with Rheumatoid Arthritis Induced by Freund Complete Adjuvant and Treated with Bee Venom. Bulletin UASVM, Veterinary Medicine 68(1).

[33] Pamuk,E.G.; Vural,O.; Turgu,B.; Demir,M.; Pamuk,O.N.&Cakir,N.2008. Increased platelet activation markers in rheumatoid arthritis: are they related with subclinical atherosclerosis.Platelets; 19(2):146-54(pubMed).

[34] Akram,M.;Shahab-Uddin;Khan, A.A.;Chani, U.;Hanan,A. ;Mohiuddin,E .& Asif, M.2010."Curcuma longa and Curcumin"-A review article. Rom.J. Biol-Plant Biol;55:65-72.

[35] Vita, J.A.& Keaney, J.F.2002.Endothelial function: a barometer for cardiovascular risk? Circulation;106:640–2.

[36] Sattar, N.; McCarey, D.W.; Capell, H.& McInnes, I.B.2003. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation;108:2957–63.

[37] Souto,A.; Salgado,E.; Maneiro,J.R.; Mera,A.; Carmona,A. & Go´mez-Reino, J.J. 2015.Lipid Profile Changes in Patients With Chronic Inflammatory Arthritis Treated With Biologic Agents and Tofacitinib in Randomized Clinical Trials. Arthritis & Rheumatology.Vol. 67,No. 1,pp 117–127.

[38] Radondi, N.;Aujesky,D.;Vittinghoff, E.;Cornuz,J.& Bauer,D.C.2006.Subclinical Hypothyroidism and the risk of coronary heart disease:A meta-Analysis. The American J. of Med.,119,541-551.

[39] AL-Maamory,F.A.D.2008.Oxidative stress and serum lipid profile in rheumatoid arthritis. Msc. Thesis. in biochemistry. College of Medicine. University of Kufa.

[40] Mansoor,A.S.&Manoj,J.2012.Anti obesity activity of Coccinia indica in female rats fed with cafeteria and atherogenic diets. Scholars Research Library Der Pharmacia Lettre,4(5):1480-1485.

[41] Mousavi, S.M.; Imani, S.; Haghighi, S.; Mousavi, S.E.& Karimi. A. et al. 2012 Effect of Iranian honey bee (Apis mellifera) venom on blood glucose and insulin in diabetic rats. J Arthropod Borne Dis; 6:136.

[42] Ivas, C. 2011. Glycemia and lipidemia variations of the rabbits inoculated with bee venom. USAMV, Romania.

[43] Singh, J.& Ranganathan, R. 2012. Quantitation of lysolipids, fatty acids, and phospholipase A2 activity and correlation with membrane polarity. J Lipid Res 53: 1993-2001.

[44] Khulan, T.S.; Ambaga ,M.& Chimedragcha, C.H.2015. Effect of Honey Bee Venom (Apis mellifera) on Hyperglycemia and Hyperlipidemia in Alloxan Induced Diabetic Rabbits. J. Diabetes Metab.6:3.

[45] Peters,M.J.; Vis,M.; van Halm,V.P.; Wolbink,G.J.; Voskuyl,A.E.; Lems,W.; Dijkmans,B.; Twisk,J. T de Koning,M. Van de Stadt,R.& Nurmohamed,M.2007. Changes in lipid profile during infliximab and corticosteroid treatment in rheumatoid arthritis. Ann Rheum Dis;66:958–961.

[46] Boers, M.; Nurmohamed, M.T.; Doelman, C.J.; Lard, L.R.; Verhoeven, A. C.& Voskuyl, A.E. et al.2003. The influence of glucocorticoids and disease activity on total and HDL cholesterol in patients with rheumatoid arthritis. Ann Rheum Dis;62:842–5.