Therapeutic effect of bee venom formulation in the treatment of FMD viral infection: Preclinical and clinical evaluation

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***Abstract*-** Foot-and-Mouth disease (FMD) is one of the world's most important infectious animal diseases especially cloven-hooved livestock caused by picornavirus and responsible for huge global losses of livestock production and trade, as well as frequent and highly disruptive large-scale epidemics. Bee (*Apis mellifera*) venom therapy is an alternative form of healing to treat various diseases. Bee venom contains at least active components which have some pharmaceutical properties; the most significant components of bee venom which have antiviral properties like mellitin, phospholipase A2 (PLA2) and Protease inhibitor. Melittin is main active component and the powerful stimulator of phospholipase A2 that destroys phospholipids and dissolves the cell membrane of the agent. Protease inhibitor inhibits the activity of different proteases like trypsin, chymotprypsin, plasmin, thrombin, thus decreasing inflammation. In this work, Egyptian bee venom (*Apis mellifera lamarckii* venom) was obtained from VACSERA used as a treatment against FMDV-O in Egypt. There are improvements in hematological, biochemical and histopathological tests, the clinical signs are decreased in the treatment period and the virus load is more significance decreases. So this study proved that the bee venom used as natural medicine has been developed to fulfill recent medical requirements without noticed side effects. Bees have been appreciated for their medicinal purposes as a treatment for many diseases and restoring the vitality of the body.

***Index Terms*-** Foot-and-Mouth Disease, FMDV-O, Bee venom, Apis *mellifera lamarckii*, hematological, biochemical and histopathological.

I. Introduction

Foot-and-Mouth Disease (FMD) is a disease listed in the World Organization for Animal Health (OIE) Terrestrial Animal Health Code. FMD is a highly contagious viral disease of cloven-hooved animals with significant economic impact, in cattle and swine as well as sheep, camels and goats as well as more than 70 species of wild animals. All species of deer and antelope as well as elephant, and giraffe are susceptible to FMD. It is caused by apicornavirus of the genus *Aphthovirus* Foot-and-mouth disease, there are seven immunologically distinct types of FMD viruses, A, O, C, SAT1, SAT2, SAT3 (South African Territories), and Asia1 (**Grubman and Baxt, 2004),** It causes damage to the epithelial cells around the mouth and feet, and the mucous membrane lining the mouth and gut system. FMD virus enters the body through inhalation. FMD morbidity is high, but mortality is usually low in adult animals. In young animal's mortality can be high because the disease damages heart muscle cells. FMD also causes painful sores and is characterized by fever and blister-like sores on the tongue and lips, in the mouth, on the teats and between the hooves. The disease causes severe production losses and while the majority of affected animals recover, the disease often leaves them weakened and debilitated (**Aftosa, F. (2014**).

Bee venom is therapy which utilizes the application of bee venom to treat many different diseases. Bee venom contains at least 18 pharmacologically active components including various enzymes, peptides and amines. It is a complex mixture of proteins, peptides and enzymes low molecular components. Using these components which have antiviral and antibacterial properties such as phospholipase A2 (PLA2), melittin, apamin, mast cell degranulating peptide, histamine, and dopamine **(Ali, 2012).** It has long been used for the treatments of chronic inflammation (rheumatoid arthritis), skin disease and for pain relief in traditional medicine for thousands of years. The aim of the present study was to determine the possible alterations in haematological and biochemical and histopathological parameters in large and small animals with FMD and under the effect of bee venom.

II. Materials & Methods

Materials: Venom: *Apis mellifera lamarckii* (Egyptian) venom. Provided from Honey Bee Keeping Department Agriculture Research Center – Egypt. **Virus:** Foot and Mouth Disease Virus (FMDV-O) Provided from Animal Health Institute –Egypt**. Animals:** Fifteen guinea Pigs (Duncken hurtly) and Twelve goats provided from Helwan Farm, VACERA – Egypt. **Blood samples:** From each animal (at time of bleeding) about 2 ml bloods were taken in vacutainers, containing EDTA. These samples were used for determining the hematological picture (erythron, leukon, and thrombon), and prothrombin time respectively. **Serum samples:** About 20 ml blood was taken simultaneously as before in vacutainers but without addition of any anticoagulant. The obtained serum was kept in a deep-freezer (at - 20°C) until used.it was used for determination of different blood chemistry, and immunological parameters.

Methods: -

Infection of tested animals by FMD virus: The test group were injected subcutaneously in the foot bad by FMDV (1×10^5dose TCID50%)in guinea pig and ($1\times10^{6.5}$ dose TCID50%) in goats, both were kept under supervision for seven days till observed the clinical signs of FMD on animals which manifested by high temperature and vesicles formation in the foot bad and between the toes.

Injection of bee venom solution as treatment in guinea pigs and goats: Guinea pigs were divided to two groups (test and control) each consist of 15 animals. The control group will be kept far from the tested animals without any infection, Application of treatment will be done just appear the vesicles by injection of bee venom 0.2 ml/animal daily intradermal by recommended dose according to the animal weight for five days. Goats were divided to two groups (test and control) each consist of 6 animals. The control group will be kept far from the tested animals without any infection. Application of treatment will be done just appear the vesicles by injection of bee venom 4 ml/animal daily intradermal by recommended dose according to the animal weight for seven days.

Collection of whole blood samples: -

Blood samples were collected from each tested animal into EDTA vacutainers. The samples were gently mixed immediately and put on ice bag till time of examination, and were used for **Hematological examination** (RBCs Count, Hematocrit value (PCV), Hemoglobin content, blood indices (MCV, MCH, MCHC, WBCs count, and platelets.

Preparation of serum samples: -

Part of the blood samples was taken simultaneously into empty clean vacutainers without anticoagulant. Each blood sample was left to coagulant at room temperature till the clot retracted. The serum was removed by automated pipette and clarified by centrifugation at 3000 r.p.m. for 15 min. And clean non hemolysed serum samples were kept at -20 °C till time of analysis to be done directly after separation. And were used for Biochemical investigations (ALT, lactate dehydrogenase (LDH), and creatinine level)

Histopathological examination: Specimens like liver, kidney, and heart from guinea Pigs and goats in all experimental groups were collected and fixed in neutral buffered formalin 10%, washed in tap water overnight and exposed to ascending concentration of ethanol (70%, 80, 90 and 100%), cleared in xylene and embedded in paraffin. Sections of the tissues (4-5 µ thick) were prepared and stained with hematoxylin and Eosin **(Bancroft *et al*, 1996).**

Statistical analysis: The obtained results were processed statistically according to **Snedecor and Cochran (1980),** where minimum, maximum, mean value, standard deviation, standard error, and range were represented. Comparison between groups for significance was done by using T-test.

III. Results & Discussion

Results: -

Preclinical evaluation of bee venom treatment against FMDV–O in guinea pigs: - Clinical signs observation: The severity of the lesion and the heels condition of the animals were noticed and monitored during the treatment period. At the seventh day of infection, we begin noticed that gradual increasing in temperature of the animals manifested by redness of the eye as seen in (Photo-2). Appearance of clear inflammation of the foot bad manifested by redness and swelling in the foot pad as shown in (photo-3), and also between the toes as seen in (photo-1). At the end of treatment period, the animals were better than previous as seen in photo (3-C), the redness on the foot pad nearly disappear and febrile condition is completely cleared as seen in photo (3-C). There are another clinical signs of FMD in guinea pigs like increasing in temperature (fever) as appeared in table (1) and reduction in the body weight as shown in table (2). Also the virus titer was determined during the treatment period and found that the titer of virus at 7th day of treatment of bee venom was decreased to be disappeared and the final titer of virus was zero as seen in table (3). Different changes are seen in hematological and biochemical analysis during the treatment period revealed improvement of the animals gradually in compare with the infected cases at the beginning of the study as seen in (table 4,5) and (figures from 1 to 2).

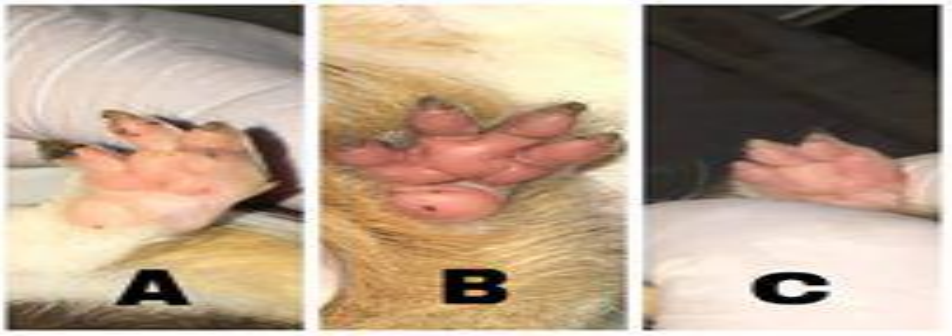

Photo (1):

A) - Shows the normal toes of guinea pig
B) - Shows the infected toes of guinea pig with swelling between the toes.
C) - Shows the treated toes of guinea pig

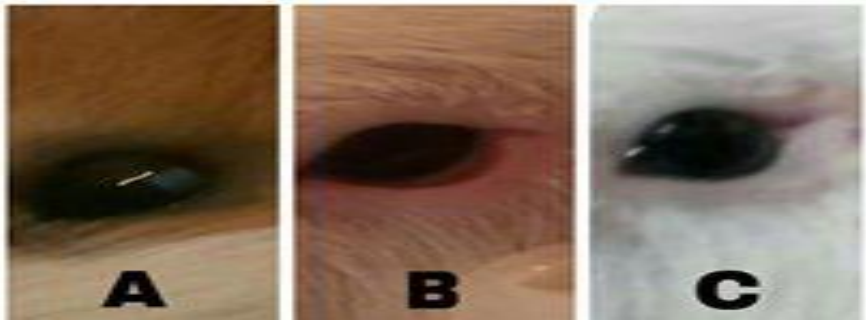

Photo (2):

A) - Shows the normal eye of guinea pig
B) - Shows the infected eye of guinea pig with redness around it
C) – Shows the treated eye of guinea pig

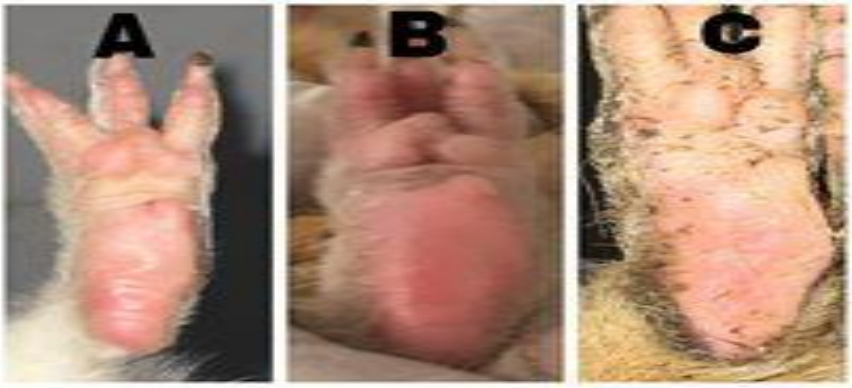

Photo (3):

A) - Shows the normal heel of guinea pig
B) - Shows the infected heel of guinea pig with Severe redness and inflammation
C) - Shows the treated heel of guinea pig

Table (1): The temperature of Guinea pigs during infection of FMDV-O and treatment of bee venom.

Normal temperature of animals	Temperature at the 7th day after infection (1st day of treatment)	Temperature of treated animals at 3th day	Temperature of treated animals at 7th day
35.9 ± 2.3 °C	38.8* ± 0.8 °C	37.5 ± 0.7°C	36 ± 0.6°C

Table (2): The body weight of Guinea pigs during infection of FMDV-O and treatment of bee venom.

Normal body weight of animals	Body weight of infected animals at 7th day	Body weight of treated animals at 7th day of treatment period
500 ± 50 gm.	460* ± 45 gm.	490 ± 49 gm.

Table (3): The titer of FMDV-O in infected and treated guinea pigs:

FMDV-O titer (infected)		FMDV-O titer (treated)			
1st day of Infection titer	At the 7th day after infection (1st day of treatment)	At 3rd day	At 7th day		
			After 24 hrs	After 48 hrs	After 72 hrs
1×10^{5}	1×10^{6}	$1\times10^{2.7}$**	$1\times10^{1.7}$***	$1\times10^{1.2}$***	0***

Hematological examination of guinea pigs: -

Table (4): Hematological values of guinea pigs: -

Parameters	Control	Test 1 (after 7 days from infection)	Test 2 (at 3rd day of Treatment)	Test 3 (7th day of treatment)
Heamoglubin (g/dI)	16.0 ±1.6	12.2 *±1.22	15.8 ±1.58	15.1 ± 1.51
Red cell count (RBCS) (millions/cmm)	5.5± 0.55	4.7±0.47	4.8±0.48	5.66± 0.566
Haematocrit (PCV) (%)	50.0 ±5	36.3*±3.63	45.0±4.3	43.0 ±4.3
MCV (fl)	90.9 ±9.09	77.2* ±7.72	89.6±8.96	85.5 ± 85.5
MCH (pg)	29.1 ±2.91	26.0*±2.6	29.2±2.9	30.0 ±3.3
MCHC (%)	32.0 ±3.2	33.8 ±3.38	32.6 ±3.26	33.7± 3.37
Platelets count (T/cmm)	333 ±33.3	310±33	316 ±31.6	313.8 ±31.38
White cell count (WBCS) (Thausands/cmm)	8.0 ±0.8	8.8 ±0.88	11.8 *±1.18	10.0**± 0.1
Staff (%)	3 ±0.3	3 ±0.3	2 ±0.2	2 ±0.2
Segmented (Neutrophils) (%)	60 ± 6	60 ±6	60 ±6	45* ±4.5
Lymphocytes (%)	30 ±3	30 ±3	30 ±3	45* ±4.5
Monocytes (%)	5 ±0.5	4 ±0.4	5 ±0.5	5 ±0.5
Eosinophils (%)	2 ±0.2	3 ±0.3	3 ±0.3	3 ±0.3
Basophils (%)	0 ±0	0 ±0	0 ±0	0 ±0

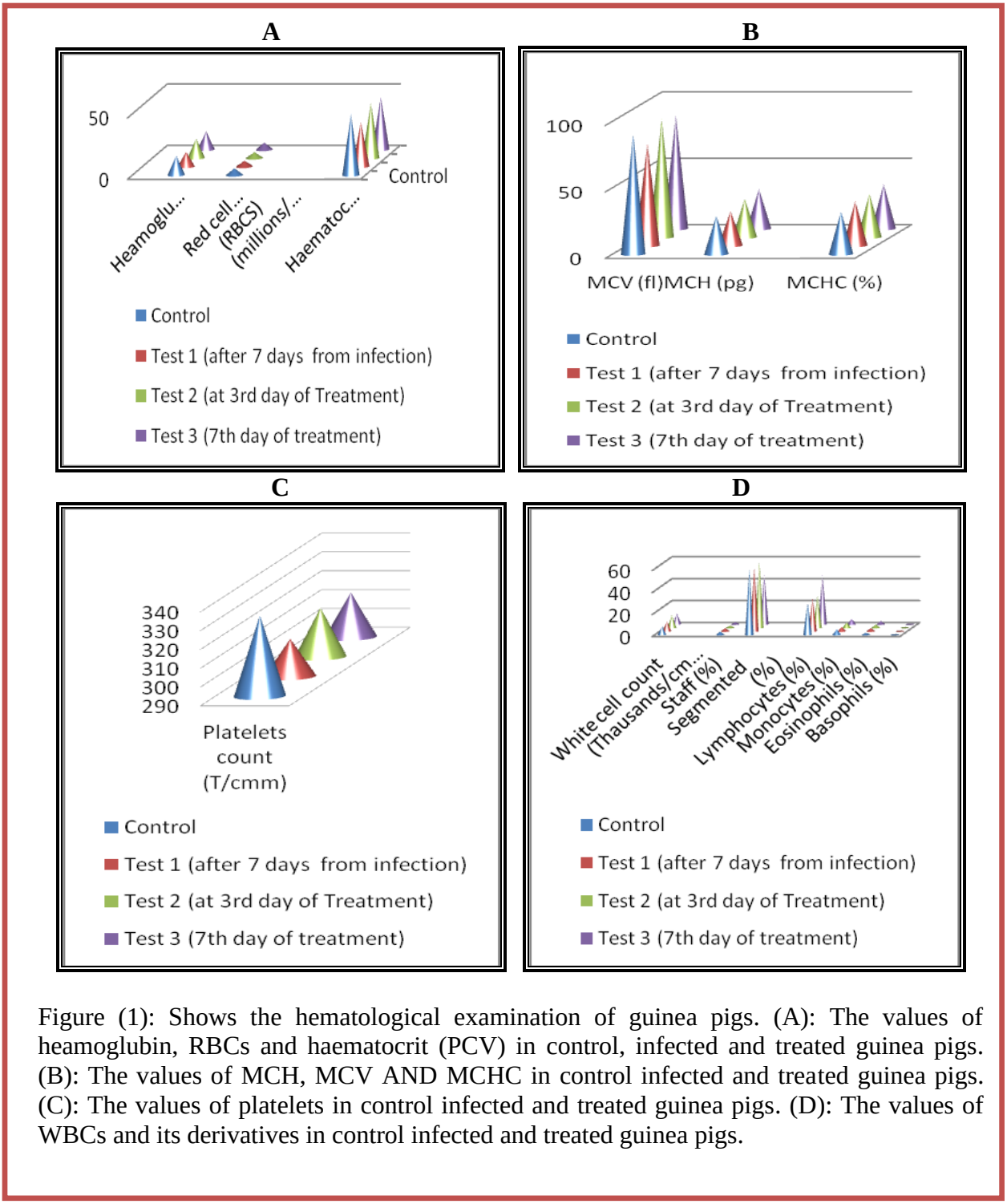

Figure (1): Shows the hematological examination of guinea pigs. (A): The values of heamoglubin, RBCs and haematocrit (PCV) in control, infected and treated guinea pigs. (B): The values of MCH, MCV AND MCHC in control infected and treated guinea pigs. (C): The values of platelets in control infected and treated guinea pigs. (D): The values of WBCs and its derivatives in control infected and treated guinea pigs.

Biochemical examination of guinea pigs: -

Table (5): Enzyme activities in serum of guinea pigs: -

Parameters	Control	Test 1 (after 7 days from infection)	Test 2 (at 3rd day of Treatment)	Test 3 (7th day of treatment)
Alanine Transaminase (ALT)(U/L)	19 ±1.9	30** ±3	26* ±2.6	21 ±2.1
S. Creatinine (mg/dl)	0.8± 0.08	1.0 ±0.1	0.7±0.07	0.7±0.07
Lactic Dehydrogenase (LDH)(U/L)	922 ±92.2	1531**± 153.1	810* ±81	683** ±68.3

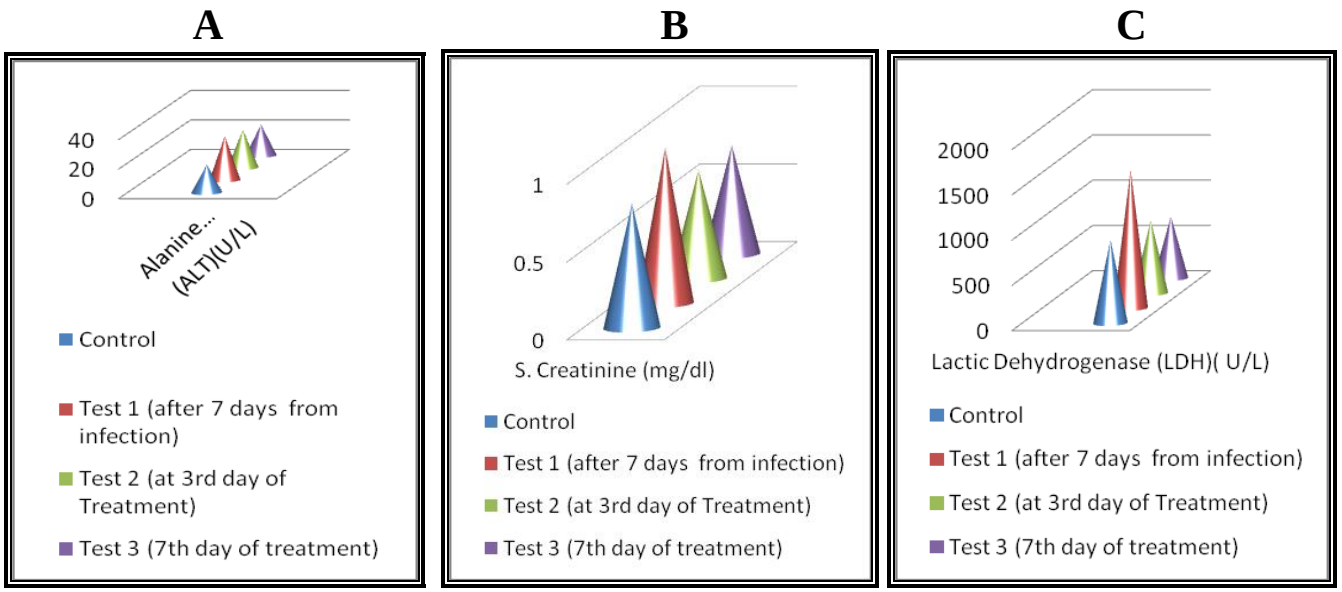

Figure (2): The biochemical values of guinea pigs.
(A): Shows ALT (AST) enzyme activity in control infected and treated guinea pigs.
(B): Shows S. creatinine enzyme activity in control, infected and treated guinea pigs.
(C): Shows LDH enzyme activity in control, infected and treated guinea pigs.

Histopathological examination of guinea pigs: -

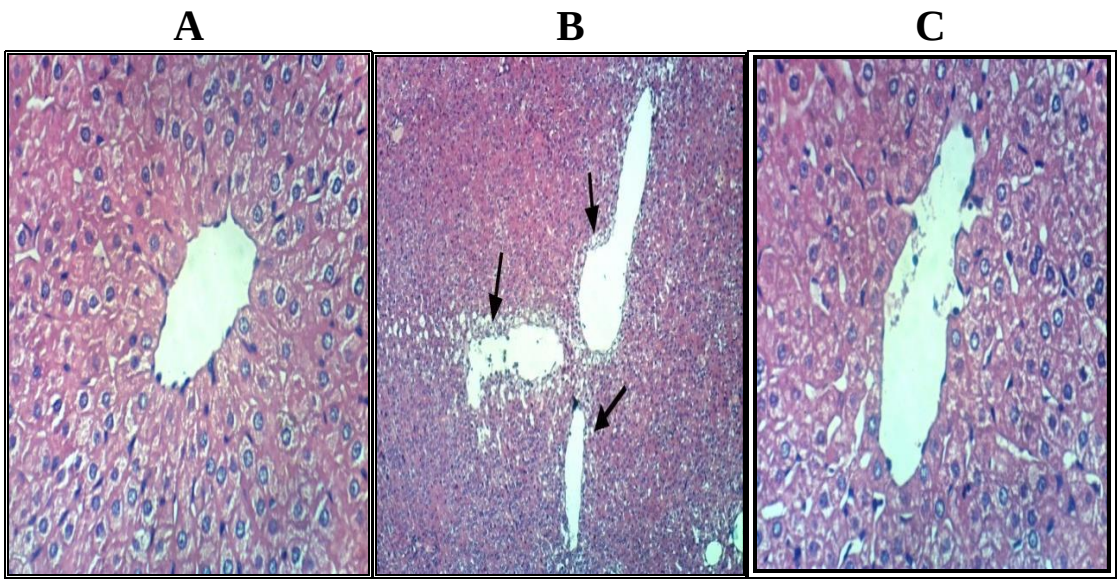

Figure (3): Represents sections of liver tissues from control, infected with FMDV-O and treated guinea pigs. (A): Shows the liver tissue of control guinea pig revealed the normal histological structure of hepatic lobule, Meanwhile, (B): Shows liver of infected guinea pig showed cytoplasmic vacuolization of centrilobular hepatocytes, while (C): Represents treated geania pig showing normalhistopathological structureof hepatic lobule (H & E X 400).

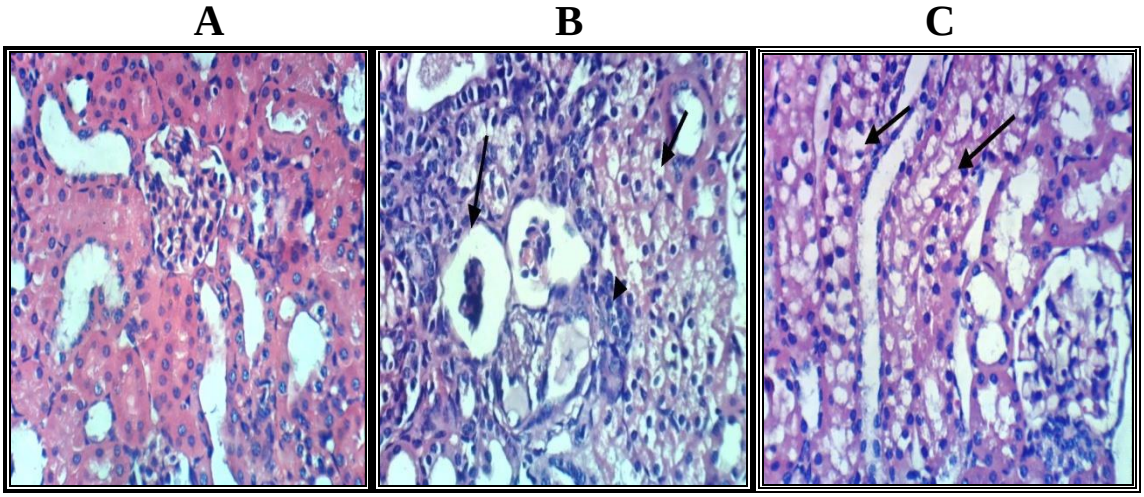

Figure (4): Sections of kidney tissue from control, infected with FMDV-O and treated guinea pigs. (A): Kidney of control guinea pig showing the normal histological structure of renal parenchyma (H & E X 400). (B): Kidney of infected guinea pig showing cytoplasmic vacuolization of epithelial lining renal tubules, atrophy of glomerular tufts and interstitial nephritis (H & E X 400). (C): Kidney of treated guinea pig showing cytoplasmic vacuolization of epithelial lining renal tubules (H & E X 400).

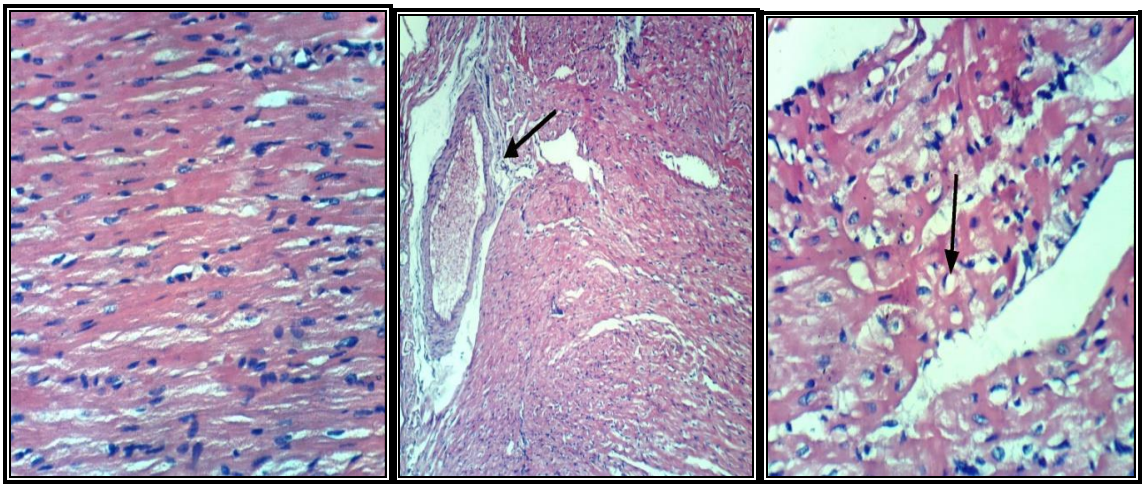

Figure (5): Sections of heart tissue from control, infected with FMDV-O and treated guinea pigs. (A): Heart of control guinea pig showing normal cardiac myocytes (H & E X 400). (B): Heart of treated guinea pig showing slightly cytoplasmic vacuolization of the sarcoplasm of cardiac myocytes (H & E X 400). (C): Heart of infected guinea pig showing perivascular oedema around the myocardial blood vessel (H & E X 100).

Clinical evaluation of bee venom treatment against FMDV–O in goats: -

Clinical signs observation: The severity of the lesion, temperature and the heels condition of the animals were noticed and monitored during the treatment period. (Photo-4) represent lesions on gum of goat, the lesions on gum nearly disappear and febrile condition is completely cleared at the end of treatment period. There are another clinical signs of FMD in goats like increasing in temperature (fever) as appeared in table (6) and reduction in the body weight as shown in table (7). Also virus titer was determined before and during the treatment period and showed that the titer of virus at 7th day of treatment of bee venom was decreased till disappeared finally as seen in table (8). Different changes are seen in hematological and biochemical analysis during the treatment period revealed improvement of the animals gradually in compare with the infected cases at the beginning of the study as seen in (table 9,10) and figures from 6 to 7.

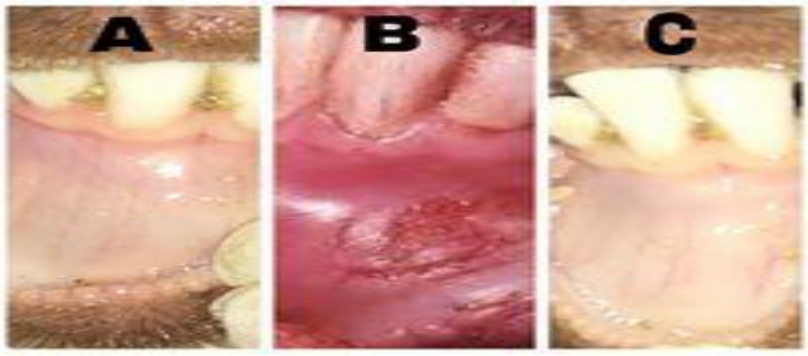

Photo (4)

A)- Shows the normal gum of goat
B) - Shows lesions appears on gum of goat
C) - Shows the treated gum of goat

Table (6): The temperature of goats during infection of FMDV-O and treatment of bee venom.

Normal temperature of animals	Temperature at the 7th day after infection (1st day of treatment)	Temperature of treated animals at 3th day	Temperature of treated animals at 7th day
38.4-40 °C Range (39.4°C)	39.9 ±3.99 °C	38.2 ±3.82 °C	38.4 ± 3.84°C

Table (7): The body weight of goats during infection of FMDV-O and treatment of bee venom.

Normal body weight of animals	Body weight of infected animals at 7th day	Body weight of treated animals at 7th day of treatment period
30 ±3.0 Kg	25±2.5 Kg	29±2.9 Kg

Table (8): The titer of FMDV-O in infected and treated goats:

FMDV-O titer (infected)		FMDV-O titer (treated)			
1st day of Infection titer	At the 7th day after infection (1st day of treatment)	At the 3rd day	At the 7th day		
			After 24 hrs.	After 48 hrs.	After 72 hrs.
$1\times10^{6.5}$	$1\times10^{6.5}$	$1\times10^{5*}$	$1\times10^{4.1*}$	$1\times10^{2.4**}$	0***

Hematological examination of goats: -

Table (9): Hematological values of goats: -

Parameters	Control	Test 1 (7th day of Infection Period)	Test 2 (3rd day of Treatment)	Test 3 (7th day of treatment)
Heamoglubin (g/dl)	11.5 ±1.15	11.4 ±1.14	9.2* ±0.92	11.3 ±1.13
Red cell count (RBCS) (millions/cmm)	5.0 ±0.1	4.4±0.4	4.56± 0.056	4.4 ±0.44
Haematocrit (PCV) (%)	33.0 ±0.3	33.9±3.39	32.2±3.22	37.6 *±3.76
MCV (fl)	30.0±3	77.0** ±7.7	79.3±3.93	76.4± 7.64
MCH (pg)	25.3±11.53	25.9±2.5	24.3±2.43	25.7 ±2.57
MCHC (%)	38.0 ±38.3	33.6 ±3.36	41.2 ±41.82	33.7±3.37
Platelets count (T/cmm)	400 ±4	979**±97.9	512* ±51.2	400.0 ±40
White cell count (WBCS) (T/cmm)	22.0 ±2.2	14.0*±1.4	13.0**±1.3	14.5* ±1.45
Staff (%)	2±0.2	2 ±0.2	1 ±0.1	2 ±0.2
Segmented (Neutrophils) (%)	45 ±4.5	55* ±5.5	50 ±5	45 ±4.5
Lymphocytes (%)	45 ±4.5	65** ±6.5	70** ±7	50 ±5
Monocytes (%)	5±0.5	5±0.5	6± 0.6	3 ±0.3
Eosinophils (%)	3 ±0.3	3 ±0.3	2 ±0.2	1 ±0.1
Basophils (%)	0 ±0	1 ±0	0 ±0	0 ±0

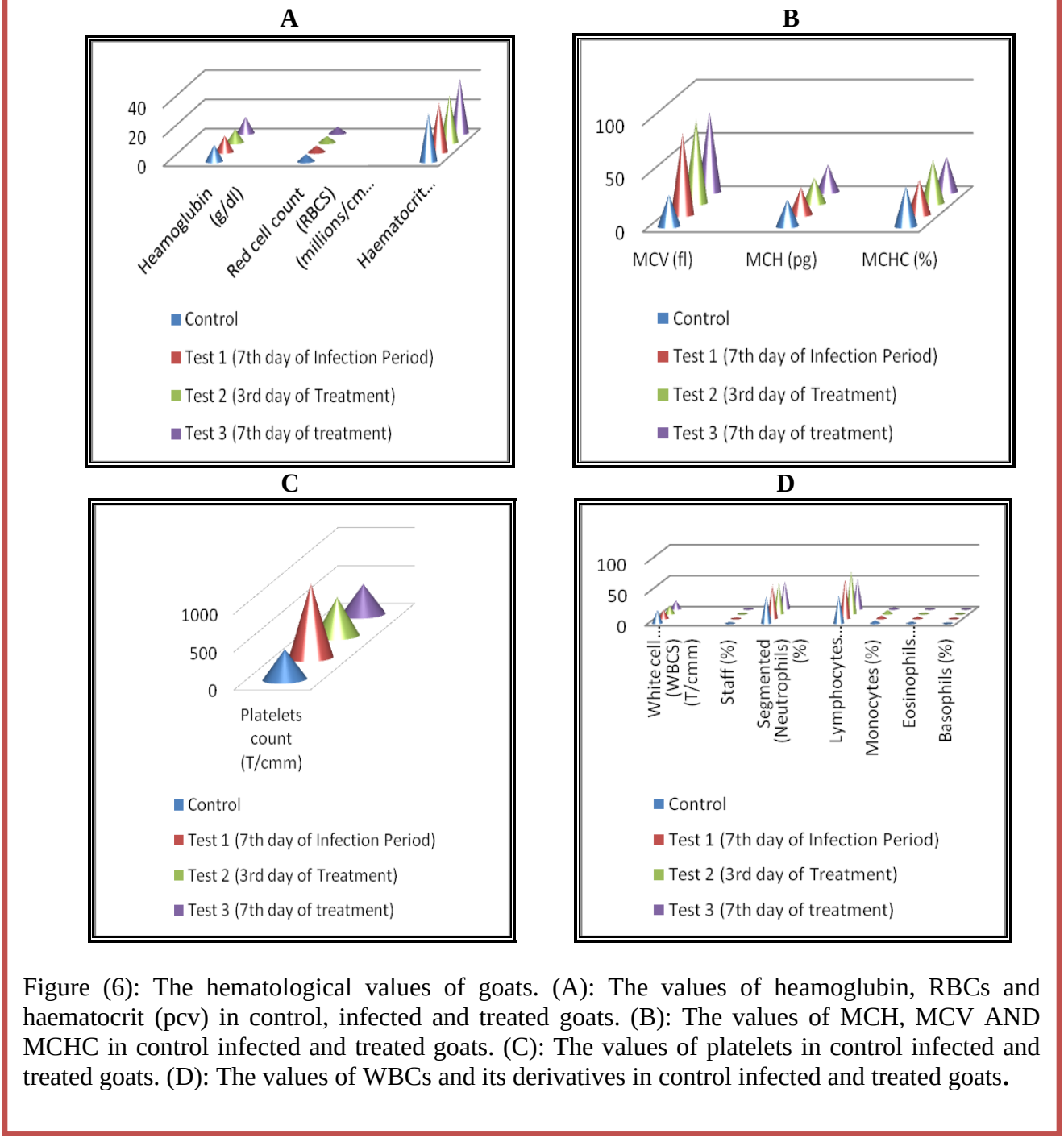

Figure (6): The hematological values of goats. (A): The values of heamoglubin, RBCs and haematocrit (pcv) in control, infected and treated goats. (B): The values of MCH, MCV AND MCHC in control infected and treated goats. (C): The values of platelets in control infected and treated goats. (D): The values of WBCs and its derivatives in control infected and treated goats.

Biochemical examination of goats: -

Table (10): Enzyme activities in serum of goats: -

Parameters	Control	Test 1 (Infection Period)	Test 2 (3rd day of Treatment)	Test 3 (7th day of treatment)
Alanine Transaminase (ALT)(U/L)	44 ± 4.4	78 *±7.8	35* ±3.5	27 **±2.7
S. Creatinine (mg/dl)	1.0 ±0.1	0.9 ±0.09	0.6 ±0.06	0.4± 0.04
Lactic Dehydrogenase (LDH)(U/L)	415 ±4.15	1224 ***±122.4	965** ±96.5	405 ±40.5

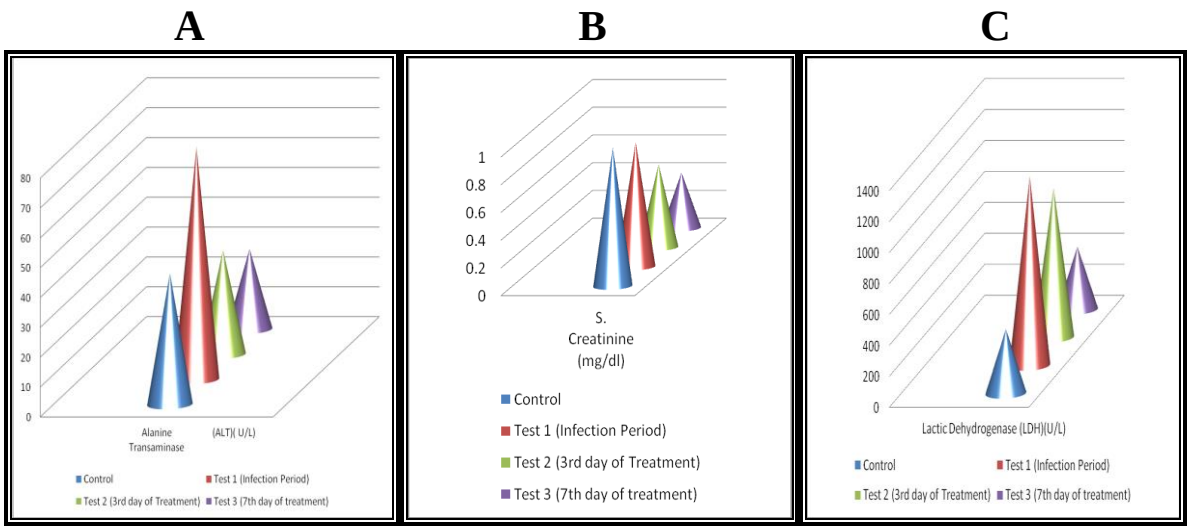

Figure (7): The biochemical values of goats.
(A): Shows ALT (AST) enzyme activity in control infected and treated goats.
(B): Shows S. creatinine enzyme activity in control, infected and treated goats.
(C): Shows LDH enzyme activity in control, infected and treated goat.

Histopathological examination of goats: -

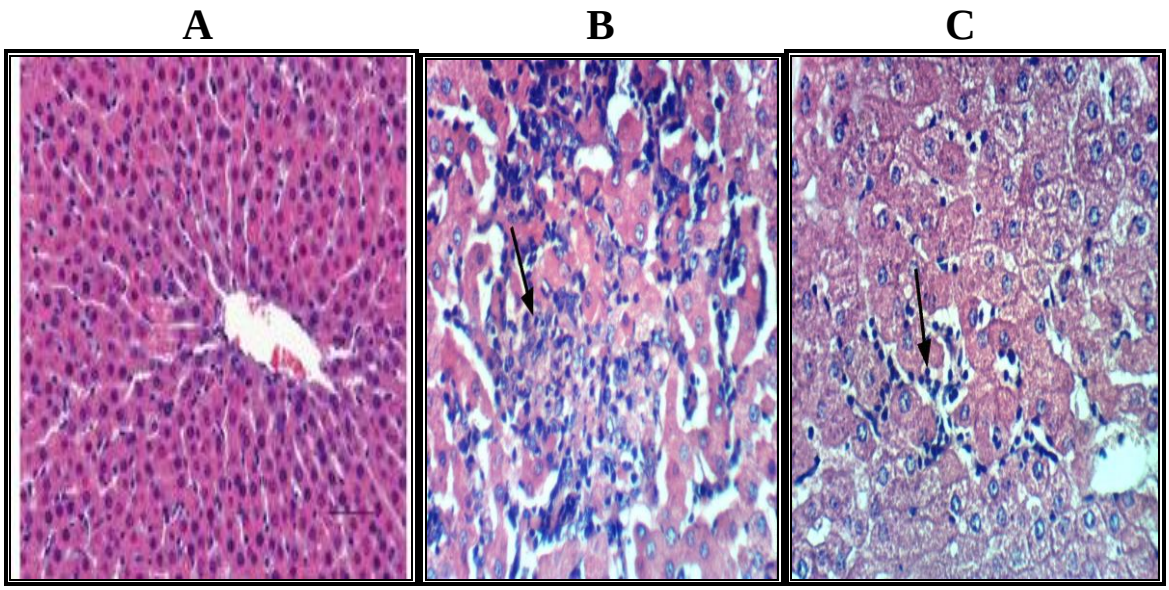

Figure (8): Sections of Liver tissue from control, infected with FMDV-O and treated goats. (A): Liver tissue of control goats revealed the normal histological structure of hepatic lobule (H & E X 400). (B): Liver infected with FMDV-O showing focal hepatic necrosis associated with mononuclear inflammatory cells infiltration (H & E X 400). (C): Treated liver showing sinusoidal leukocytosis (mainly lymphocytes) (H & E X 400).

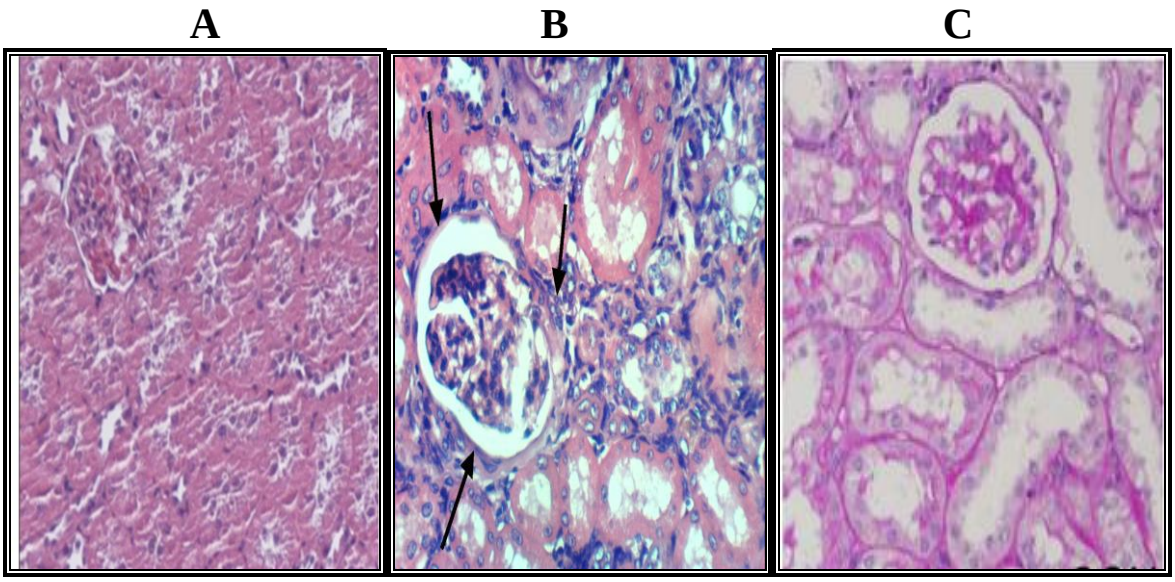

Figure (9): Sections of Kidney tissue from control, infected with FMDV-O and treated goats. (A): Kidney of control goats showing normal cardiac myocytes (H & E X 400). (B): Photomicrograph of kidney infected with FMDV-O showing interstitial nephritis and thickening of the parietal layer of Bowman's capsule (H & E X 400). (C): Treated kidney showing cytoplasmic vacuolization of epithelial lining renal tubules (H & E X 400).

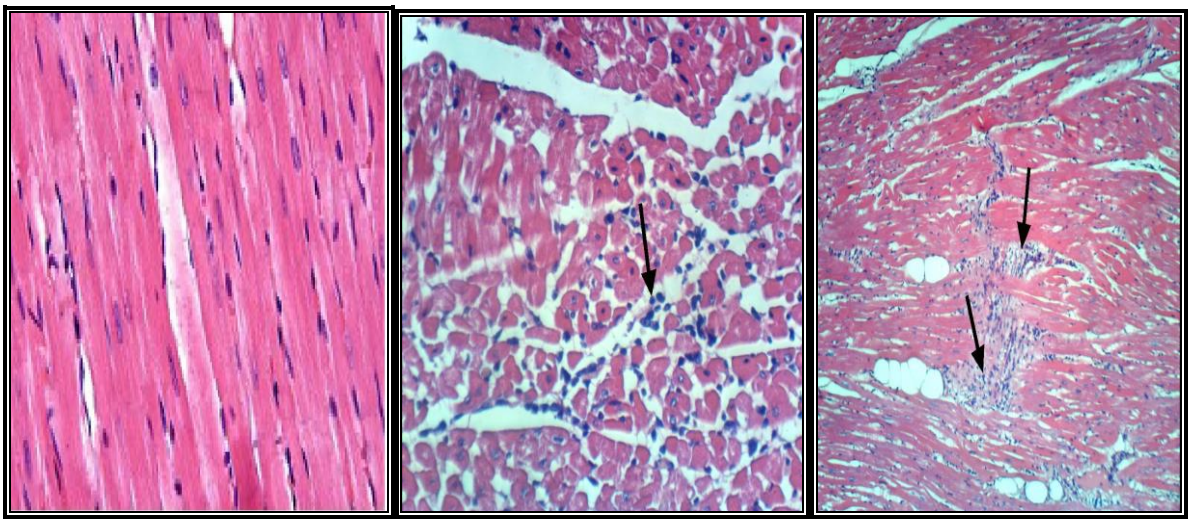

Figure (10): Sections of heart tissue from control, infected with FMDV-O and treated goats. (A): Heart of control goats showing normal cardiac myocytes (H & E X 400). (B): Photomicrograph of heart infected with FMDV-O showing focal Zenker's necrosis of myocytes associated with mononuclear cells infiltration (H & E X 400). (C): Treated heart showing intermuscular mononuclear inflammatory cells infiltration (H & E X 100).

Discussion: -

Regarding to the previous results, Infection of Guinea pigs by FMDV-O resulted in redness and swelling in the foot-pad and eye and between the toes and febrile /condition, these previous results due to the effect of virulent virus on guinea pig as agreed by **Chen and Liu (2013)**, **Lohse, *et al* (2012)**, **Wang *et al*, 2011**, **Núñez *et al*, 2007**, and **Knudsen *et al*, 1979.** Infection of goats by FMDV-O resulted in appearance of lesions in gum and febrile condition of the animals manifested by shivering in all tested animals, these previous results due to the effect of virulent virus on goats and guinea pigs as agreed by **Gakuya *et al* (2011), and Wernery & Kinne (2012), while rectal** temperatures was determined and we found significant decrease in temperature after treatment by bee venom injection as shown in (table 1,6), this result was agreed with (**Kittelberger, *et al*, 2015).** Regarding the hematological results, total number of RBCs in the infected group was significantly lower than that in the treated group ($P < 0.05$), while MCV values were significantly higher in the treated group. There were no significant differences in the other haematological parameters between the groups. The significant reduction in RBCs and elevation in MCV may be attributed to endocrinopathy as reported previously by **(Gokce *et al.*, 2004)**. Regarding the biochemical results as shown in (5,10), there were significant increase in ALT, Creatinine and LDH in infected group comparing with the control group as reported previously by **(Gokce *et al.*, 2004**)., these biochemical and haemotological alterations may indicate the development of anaemia and pancreatic dysfunction in animal suffering from FMD. This study also indicated that Aphtovirus induces the production of nitric oxide, in vivo. These findings provided information on our understanding of the clinical pathology and pathogenesis of FMD.

Treatment of diseased guinea pigs and goats by bee venom injection daily for 7 days resulted in disappearance of the previous clinical signs at the end of treatment period.

Regarding the RBCs count, hemoglobin content, and PCV value of infected and test guinea pigs and goats as shown in (tables 4, 9 and figures 1 and 6), there was non-significant increase in RBCs count, and hemoglobin content after 7 days of bee venom injection with concomitant gradual significant increase in Hematocrit percent within normal range along the period of the study. Similar results obtained by **(Husseien, *et al*, 2001).** While MCV, MCH and MCHC values showed no significant increase along the testing period between the infected and tested group. This result was in agreement with that previously reported by (**Ishay *et al*, 1975).** The overall results of the erythron indicated that injection of bee venom in guinea pigs for 7 days resulted in improving blood status which explained by improving integrity of RBCs leading to increase in erythron and stimulatory effect on the heart that may result in increasing blood flow as reported by **(Vick *et al*, 1974), (Eliezer kaplinsky *et al*, 1976**) and **Husseien *et al* (2001).** Concerning leukons, there was significant increase in total leukocytes with concomitant increase in lymphocytes count although this increase was within the normal range after 7 days of bee venom injection compared with the control group. This may be due to enhancement of lymphocytosis under the effect of bee venom components as PLA2 which discussed by **(Prinz *et al*, 1987)**, or due to lymphocytes proliferation by stimulation of immune system as result of bee venom injection, this was assessed by **(Lomnitzer and Rabson, 1986).** Monocytes showed non-significant decreased after 7 days of bee venom injection although this was within the normal range. This decrease can clarify that increase in lymphocytes was due to its proliferation and not due to allergy

otherwise monocytes was also increased in response to allergy **(Moneret -Vautrin *et al*, 1987).** Neutrophiles showed non-significant decreased **while** esinophiles and basophiles count cause non-significant changes along the test period, these results were in agreement with **(Somerfield *et al*, 1984)** and may be due to the injection of nontoxic dose as mentioned by **(Kosnik and Wraber, 2000).**

The platelets count as shown in (table 4, 9 and figures 1,6), there was non- significant increase after 7 days of bee venom injection, while the observed increament in the values were within the normal level. This platelets activity was due to bee venom hypersensitivity **(Tsicopolous *et al*, 1989)** but the use of bee venom in special schedule for injection may cause desensitization which give rise to adjustment of platelets level to be in the normal range. Also may be due to inhibition in platelets aggregation under the stimulatory effect for production of prostaglandin as mentioned by **(Ribardo *et al*, 2001)**.

Regarding the biochemical activities, AST activity showed moderate significant decrease ($P \geq 0.01$) in its levels in treated group along the test period as shown in (table 5,10 and figure 2, 8). These give us more confidence that bee venom has improvement action on the liver. The serum creatinine level (table 5, 10 and figure 2, 8) of treated group resulted in significant decreases after 7 days with no-significant decrease along the test period compared with infected group. This means that bee venom has benefit effect on the kidney. As shown in (table 5, 10 and figure 2, 8), there was moderate significant decrease in the LDH levels in compared with infected group along the test period. These previous biochemical changes attributed to the benefit effect of bee venom on the blood which increase the tissue tolerance to lack of oxygen (hypoxia) with increase in arterial blood flow and vascular permeability so the exchange between blood and tissue is increased **(Foster, 1969, Vick *et al*, 1974, and Chkenderow and Kobourova, 1981).** This explained that bee venom didn't induce cellular toxicity determined by decrease in release of lactic dehydrogenase (LDH).

Regarding the pathological examination of guinea pigs and goats, as shown in (figure 3,4,5,8,9,10), Microscopically, In Figure (3-B) shows liver of infected guinea pig showed cytoplasmic vacuolization of centrilobular hepatocytes, while figure (3-C) represents treated geania pig showing normalhistopathological structureof hepatic lobule. In Figure (4-B) shows sections of kidney tissues of infected guinea pig revealed cytoplasmic vacuolization of epithelial lining renal tubules. Meanwhile, In figure (4-C) shows treated geania pig revealed slightly vacuolization. In figure (5-B) shows heart of infected geania pig showing perivascular oedema around the myocardial blood vessel. Meanwhile, In figure (5-C) represents heart of treated guinea pig showing slightly cytoplasmic vacuolization of the sarcoplasm of cardiac myocytes. In figure (8-B) shows liver infected with FMDV-O showing focal hepatic necrosis associated with mononuclear inflammatory cells infiltration. In figure (8-C) shows treated liver showing sinusoidal leukocytosis. (9-B) represents Photomicrograph of kidney infected with FMDV-O showing interstitial nephritis and thickening of the parietal layer of Bowman's capsule and in figure (9-C) shows treated kidney showing cytoplasmic vacuolization of epithelial lining renal tubules. In figure (10-B) shows heart infected with FMDV-O showing focal Zenker's necrosis of myocytes associated with mononuclear cells infiltration. (10-C) shows treated heart of goat showing intermuscular mononuclear inflammatory cells infiltration. Finally, there was improvement in histopatholgical case according to the previous results comparing between treated and infected organs.

By determination of the virus load before and after the treatment period we found that there is significant decrease in virus load along the period of treatment in compare with the beginning virus load. These results revealed that there is antiviral activity of *Apis mellifera lamarckii* venom may be due to the high level of meliittin and These results were agree with **De-Clercq *et al*, (2000)** proved that bee venom melittin inactivates HIV and also aborts cell-to-cell fusion and transmission of HIV, due to its high-affinity interaction with gp120 and **Masuda *et al*, (2005)** explained that the antiviral effect of the bee venom phospholipase A2 depends essentially on its ability to hydrolyze phospholipids in host cell membranes Also proved that Bee venom melittin inactivates HIV and also aborts cell-to-cell fusion and transmission of HIV, due to its high-affinity interaction. As shown in **Moreno and Giralt (2015)**, The antiviral activities described for melittin and its analogs are caused by specific intracellular events, with the selective reduction of the biosynthesis of some viral proteins, as reported for the melittin analog Hecate on herpes virus-1 and for melittin itself on HIV-1-infected lymphoma cells. In the 90s, active melittin was presented to provide an improved composition complementary to azidothymidine (AZT) to inhibit the reverse transcriptase and growth of HIV-infected cells. Another study of **Bose and Acharya (2015)** shown that although melittin destroys the infectivity of HIV particles, the utility of this toxin is limited by its nonspecific cytotoxic effects: melittin kills cells by disrupting membrane structure and function. **Lawrence and Skalka (1980)** stated that the melittin compound can make the viral envelope permeable, that make it loss of its infectivity and **Mitsuishi *et al*, (2006)** indicated that the direct addition of bee venom phospholipase A2 to 293A cells suppressed adenovirus plaque formation in both number and size. **Pharney (1996)** described the beneficial effect of bee venom active components on the human body as follow: Apamin and melittin were more potent than penicillin against certain bacteria, while mast cell degranulating peptide and adolapin were non-steroidal anti-inflammatory. **Matsuzaki (1997)** said that honeybee venom contains at least 18 active substances. Melittin, is the most prevalent and it is one most potent anti-inflammatory agents known (100 times more potent than hydrocortisol). Adolapin is another strong anti-inflammatory substance and inhibit cyclooxidase. Apamine inhibits complement C3 activity and blocks calcium-dependent potassium channels, thus enhancing nerve transmission. Other substances such as compound X, hyaluronidase, phospholipase A2, histamine and mast cell degeneration protein are involved in the inflammatory response of the venom in during a softening of tissue and facilitation of fluid flow.

These results due to **antiviral effect of bee venom** as reported by**De-Clercq *et al*, (2000), Masuda *et al*, (2005), Moreno & Giralt (2015)**, **Bose and Acharya (2015)**, **Lawrence and Skalka (1980)**, **Mitsuishi *et al*, (2006), Ramadan *et al*,**

(2009), **(Fenard *et al,* 1999)**, **(Yong *et al,* 1990) and (Yunginger *et al,* 1978)** whose mentioned that this antiviral activity due to melittin and or phospholipase A2 enzyme activity also may be due to protease inhibitor which involved in bee venom (**Bogdanov, 2015).** Also bee venom **has anti-inflammatory** effect which manifested by decrease foot bad inflammation during the treatment period by induction of COX2 expression and stimulation of prostaglandin generation which have biological activities resulting in revealed viremia and inflammation as reported by **Ribardo *et al,* (2002), Park *et al* (2004)**, **kim (1992)** and **lee *et al,* (2005)**.

Also in **Vick *et al,* (1972)** observed that bee venom or one of its components (melittin) when injected S/C in Rhesus monkeys stimulated the production of cortisol from the adrenal gland through its action on the pituitary gland without any gross or microscopic tissue changes. These observations may explain bee venom benefits in variety of disease conditions that also respond to adrenal steroid therapy. In **Lee *et al,* (2004)** study reported that acupuncture therapy with bee venom suppressed the development of arthritis and caused inhibition of the immune responses in type-II collagen-induced arthritis. The incidence of arthritis, the mean arthritis index, and the number of arthritic limbs were significantly lower in the bee venom treatment compared to the control group. Among the serum pro-inflammatory cytokines, examination of the histopathology of the joints of murine CIA showed decreased inflammation signs and less lymphocyte infiltration after bee venom acupuncture therapy. **Hyoung *et al,* (2005)** stated that bee venom has been used traditionally for the control of pain and inflammation in various chronic inflammatory diseases. They also suggested that bee venom inhibits the proliferation of rheumatoid synovial fibroblast cells through induction of apoptosis by caspase-3 activation. **Kwon *et al,* 2003** reported that subcutaneous bee venom injection produced a marked suppression of leucocyte migration and tumor necrosis factor (TNF)-alpha concentration. The anti-inflammatory effect bee venom administration was mediated in part by the release of catecholamines from the adrenal medulla. Also the previous results may be attributed to effect of bee venom **as immunomodulator** which it stimulates immune system to protect the body against infection by its stimulation of prostaglandin generation which have biological activities resulting in revealed of viremia due to stimulation of IL-10, TNF alpha, and CD8 which result in regulation in IgE which responsible for histamine release **(Rekka *et al,* 1990**). **McHugh *et al,* (1995)** examined changes in cytokine secretion in patients before and during both rush and conventional venom immunotherapy (VIT) in bee venom allergen patients and showed that immunotherapy shifted cytokine responses to allergen from a TH-2 to a TH-1 dominant pattern, suggesting direct effects on T cells. So it is possible to use whole venom in treatment of various medical disorders/diseases. **Kammerer *et al,* (1997)** said that venom immunotherapy (VIT) lead to transient increase in T-cell proliferation followed by T-cell hypo responsiveness and modulation of cytokine secretion from T (H0)-type to a T(H1) type pattern. **Akdis *et al,* (1998)** found that bee venom phospholipase A2 (PLA) induced high IL-4, IL-5 and IL-13 production in peripheral blood mononuclear cell cultures. These features may enable new applications for safer immunotherapy.

IV. Recommendation

From the previous project we recommend to use bee venom as injection as alternative medicine in veterinary practice as it is safe and effective medication against virus infection specially RNA viruses as seen in the previous results on FMDV-O. We succeeded to treat laboratory and large animals by bee venom. Using of bee venom by intradermal injection is useful to maintain the health condition through its immune-stimulant effect.

Acknowledgment

The authors have taken efforts in this project; however, it would not have been possible without the kind support and help of many individuals. The authors extremely thankful and indebted to Dr. Gehan Safwat for sharing expertise, and sincere and valuable guidance and encouragement extended to me. Various members of VACSERA team also provided considerable assistance during the project. Finally, I want to thank Dr. Samia Kamal for providing the Foot-and-mouth disease virus Type-O from Animal Health Institute –Egypt**.**

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