

Apis mellifica — An Effective Insect Drug

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Abstract

Apis mellifica (Syn: *Apis mellifera*) belonging to family Apidae. Its extract contains melittin, glycosidase, hyaluronidase, phosphatase α -glucosidase, phospholipase A2, phospholipase B, protease inhibitor, apamine, adolapine, secapine, minimine, histamine, tertiapin, dopamine, mast cell degranulating peptide, pamine, procamine A, noradrenalin, γ -aminobutyric acid, α -amino acids, glucose, fructose, complex ethers, potassium, calcium, magnesium. *A. mellifica* extract was made from whole insect. It is used in bites, stings, sore throat, urine retention, pain and headaches. There are no documented side effects associated with it.

Keywords

Honey bee, Homoeopathy, Anti-inflammatory.

1. INTRODUCTION

Apis mellifica is approximately 15 mm long, shiny black, covered with orange-red and grey hair insect with broad spineless tibiae. The posterior margins of the segments and legs are brown, with gradual transition to yellowish-red. Maxillary palps are single-membered and claws are two membered, while hind limb has bristles. There are three complete cubital cells in the wing along with the radial cells. A duct connects

the barbed sting with the poison sac (Raes *et al.*, 1985; Tsujiuchi *et al.*, 2007; Farah-Saeed and Ahmad, 2016a).

Historical Background and Medicinal Uses

Bee products medicinal uses have been described in ancient literature by Hippocrates, Aristotle and Galen. Bee venom was used in middle ages as a medical remedy to relieve pain and to treat inflammatory diseases such as arthritis and rheumatism. The use of *A. mellifica*, a whole honeybee was discovered by Rev. Brauns in 1835 as a homeopathic remedy, in Thuringia, Germany. Dr. Constantine Hering, in 1853, published the evidences of *A. mellifica* efficacy in his American Provings. The safety and effectiveness of *A. mellifica* has made it a popular homeopathic remedy (Dunglison 1856; Urtubey 2003; Hellnrm *et al.*, 2008; Hering 1879; Simics, 2003).

Preparation of A. mellifica Extract/Tincture

Live honey bees are placed in a clean, wide-mouthed suitable container, preferably of glass. After mincing and shaking, the menstrum is poured in, and macerated for fourteen days, swirling three times daily. The mother tincture obtained is filtered. It is important that the bees should not be pressed. It is repeatedly diluted

Table 1a: Chemical Constituents Activities of *A. mellifica* L.

S.No.	Chemical constituents	References
1.	Melittin	Banks and Shipolini, 1986; Dotimas and Hider, 1987; Shkenderov and Ivanov, 1983; Han <i>et al.</i> , 2010; Muli and Maingi, 2007; Urtubey, 2005
2.	Hyaluronidase	
3.	Phosphatase α -glucosidase	
4.	Phospholipase A2 (PLA2)	
5.	Phospholipase B	
6.	Apamine	
7.	Adolapine	
8.	Secapine	
9.	Minimine	
10.	Glycosidase Tertiapin	
11.	Mast cell degranulating peptide	
12.	Pamine	
13.	Procamine A	
14.	Protease inhibitor	
15.	Noradrenalin	
16.	Histamine	
17.	Dopamine	
18.	γ -aminobutyric acid	
19.	α -amino acids	
20.	Glucose	
21.	Fructose	
22.	Complex ethers	
23.	Potassium	
24.	Calcium	
25.	Magnesium	

Table 1b: Pharmacological Activities of *A. mellifica* L.

Pharmacological actions	References
Rheumatism, Cancerous tumors, Multiple sclerosis, Dementia, Post stroke paralysis, Polyneuritis, Ganglion nerve inflammation, Neurodynia, Cerebellar ataxy, Syringomyelia, Inflammation of facial nerve, Myopathy, Trigeminal neuralgia, Post-traumatic inflammation of plexus nerve, Inflammation of arachnoid CNS membrane, Parkinson, Arteriosclerosis, Endarteritis, Angina pectoris, Arrhythmia, Eczema, Dermatitis, Psoriasis, Furunculosis, Healing of cicatrices, Baldness, Colitis, Ulcers, Asthma, Bronchitis, Pharyngitis, Tonsillitis, Ear nerve neuritis, Polyarthritus, Osteochondrosis, Hypertension, Urinary retention, Edema, and dropsy. In homeopathy for treatment of: inflammatory diseases of eyes, ears, respiratory organs, Diseases of digestive system α -bladder, kidney; Skin diseases, allergies, acne, abscesses; Scarlet and German measles, diphtheria, Glandular and genital diseases, Heart and blood circulation diseases; Psychiatric diseases, Kidney inflammation, Urine suppression and general edema.	Ellingwood, 1919; Kokot and Matysiak, 2009; Krylov, 1995; Krylov <i>et al.</i> , 2007 Ludyanskii, 1994; Pinto, 2009; Putz <i>et al.</i> , 2006; Russell <i>et al.</i> , 2004; Savilov, 2010; Schraner, 2007; Shkenderov and Ivanov, 1983; Son <i>et al.</i> , 2007; Urtubey, 2005.

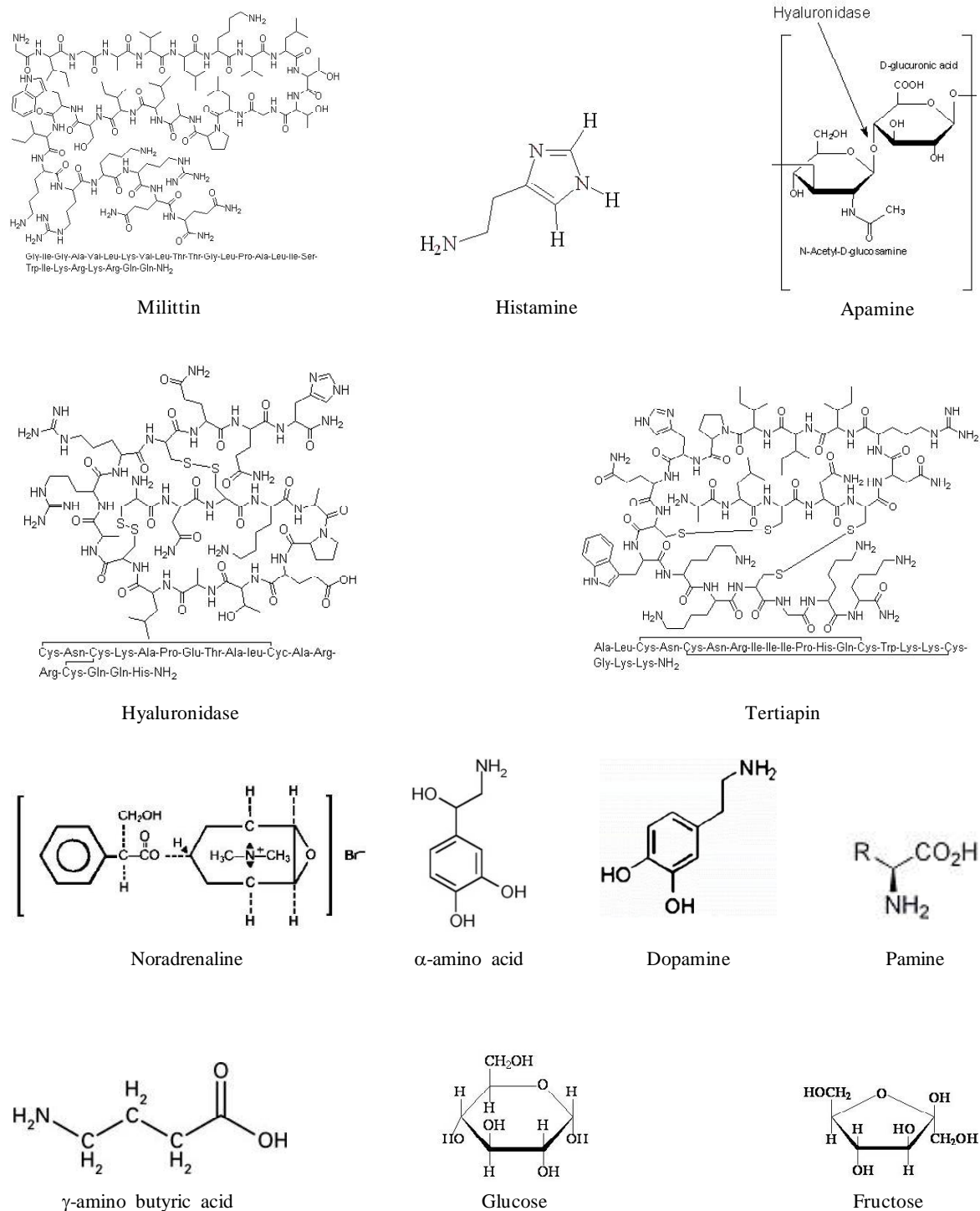


Fig. 1
 Active chemical constituents of *Apis mellifica*

Table 2: Active Constituents in *A. mellifica* L. with their Therapeutic Efficacy

S.No.	Active constituents	Therapeutic efficacy	References
1.	Melittin	Increases capillary permeability, lowering the blood pressure, decrease total cholesterol and triglyceride but increased HDL in serum, lowers blood coagulation. Immuno-stimulatory and immuno-suppressive, radioprotective, influences the central nervous system, improved motor function and inhibit neuronal death in the spinal cord, anti-cancer, increase cell viability and decrease apoptosis, anti-bacterial, anti-fungal and anti-viral. Reduce swelling and granulomatous responses. Anti-arthritic, anti-inflammatory effect for liver effects	Shkenderov and Ivanov, 1983; Urtubey, 2005; Son <i>et al.</i> , 2007; Asafova <i>et al.</i> , 2000; Jeong <i>et al.</i> , 2011; Ludyanskii, 1994; Krylov, 1995; 2007; Savilov, 2010, Lee <i>et al.</i> , 2014; Han <i>et al.</i> , 2014; Kim <i>et al.</i> , 2011; Park <i>et al.</i> , 2012; 2014
2.	Phospholipase A	Destroys phospholipids and dissolves the cell membrane of blood cells; lowers the blood coagulation and blood pressure, prevents neuronal cell death caused by prion peptides	
3.	Phospholipase B	Detoxicating activity	
4.	Hyaluronidase	Catalyses the hydrolysis of protein, dilates blood vessels, increases their permeability, causing increased blood circulation	
5.	Apamine	Anti-inflammatory, increases the defense capability, immune-supressor and stimulates the CNS	
6.	MCD (Mast cell degranulating peptide)	Anti-inflammatory effect, stimulates CNS, lyses mast cells, releasing histamine, serotonin and heparin, melittin-like effect increasing capillary permeability	
7.	Adolapin	Inhibits the specific brain enzymes, cyclo-oxygenase and lipooxygenases, anti-rheumatic, decreases pain, anti-pyretic, inhibits the aggregation of erythrocytes	
8.	Protease inhibitor	Inhibits the activity of different proteases, thereby reducing inflammation and act as anti-rheumatic	
9.	Cardiopep	Radioprotective	
10.	Minimin		
11.	Procamine		
12.	Histamine	Dilates blood vessels, increasing the permeability of blood capillaries and increase blood circulation, stimulates smooth muscles	

till the virulent aspect of the bee venom is removed, leaving only the curative agent. As a consequence it becomes an effective potent medicine for an actual honeybee sting and for ailments that have similar symptoms (*British Pharmacopeia*, 2015).

A) Physico-chemical Examination

Relative Density

According to *British Pharmacopoeia* (Vol. IV) the relative density of *A. mellifica* extract is 0.890-0.910 (*British Pharmacopeia*, 2015).

Dry Residue

According to *British Pharmacopoeia* (Vol. IV) the dry residue is not less than 1.25 and not more than 1.60% (*British Pharmacopeia*, 2015).

Chemical Identification

In reaction with chemical reagents the extract of *A. mellifica* extract showed the presence of tannins, saponin glycosides, carbohydrates, and steroids (Farah-Saeed, 2014).

Spectroscopy (FTIR)

A. mellifica revealed significant peaks at 3235.87 (OH), 2937.57 (C-H), 1621.79 and 1519.64 (aromatic ring), 1050 (C-O-C) cm^{-1} (Farah-Saeed and Ahmad, 2016a; Kokot and Matysiak, 2009).

B) Pharmacological Activities

Insecticidal

Paralysis effect and mortality was seen with the increase in dose. Exposure to 100 mg of *A. mellifica*, mean mortality time was 10.6 ± 2.60 hours (Farah-Saeed, 2014).

Anthelmintic

A. mellifica administered in the snails at 1 mg, 25 mg, 50 mg, 75 mg and 100 mg

respectively, did not reduce its activity even after 72 hours. However, at 500 mg of the drug, time dependent decrease of activity with shell discoloration was observed. At 1000 mg, time dependent decrease in activity was also observed, indicating that at the end of 72 hours out of 6 snails, 2 (33%) had paralytic effect with shell discoloration (40 ± 5.54), whereas 4 were found to be dead with 60% mortality (Farah-Saeed, 2014).

Anti-bacterial

A. mellifica did not exhibit zone of inhibition against bacterial or fungal pathogens. No minimum inhibitory concentrations (MIC) against bacterial pathogen was observed against the tested pathogens (Farah-Saeed, 2014).

Al-Ani *et al.*, (2015) investigated the antimicrobial activity of bee venom and its main component, melittin, single or in double-drug and triple-drug combinations with antibiotics (vancomycin, oxacillin, and amikacin) or antimicrobial plant secondary metabolites (carvacrol, benzyl isothiocyanate, the alkaloids sanguinarine and berberine) against drug-sensitive and antibiotic-resistant microbial pathogens. The secondary metabolites were selected corresponding to the molecular targets to which they were directed, being different from those of melittin and the antibiotics (Issam *et al.*, 2015).

Anti-oxidant

A. mellifica (1 mg) exhibited 88.93% DPPH scavenging activity and 82.98% total anti-oxidant activity by phospho molybdate method (Farah-Saeed, 2014).

Neuro-pharmacological

The anxiolytic activity was assessed using open field, head dip, light and dark, cage cross and swimming apparatus. The CNS depressive effects were observed at 100 mg/kg of *A. mellifica* extract as follows; in open field

Table 3: Identification of Chemical Constituents of Drug *A. mellifica* by Chemical Reagents using Thin-layer Chromatography

Chemical constituents	Chemical reagents	Reaction
Tannins	Lead acetate	Very slight precipitation
Saponins		Froth formation
Alkaloids	Dragendorff's reagent	No orange precipitates
Carbohydrates	Molish test	Color changes to purple
Proteins	Ethanol	Soluble
Sterols	Lielarmann Burchardt reagent	No Purple ring formation
Steroids	Napthol-H ₂ SO ₄	Purple color which on addition of water remains purple
	H ₂ SO ₄	Golden brown

TLC of *A. mellifica* extract in chloroform – methanol – water (80:20:2) solvent system displayed the presence of several spots of different chemical classes. The R_f values of the spots were: 0.02, 0.10, 0.15, 0.23, 0.35, 0.45, 0.66 at 254 nm and 0.03, 0.09, 0.21, 0.43, 0.50, 0.0.73, 0.78, 0.87 at 366 nm. In ethyl acetate – methanol – water (100:16.5:13.5), the R_f values of the spots were: 0.01, 0.09, 0.23, 0.38, 0.46, 0.53, 0.59 at 254 nm and 0.03, 0.05, 0.11, 0.16, 0.22, 0.26, 0.39, 0.46, 0.52 at 366 nm. (Farah-Saeed and Ahmad, 2016a; Kokot and Matysiak, 2009).

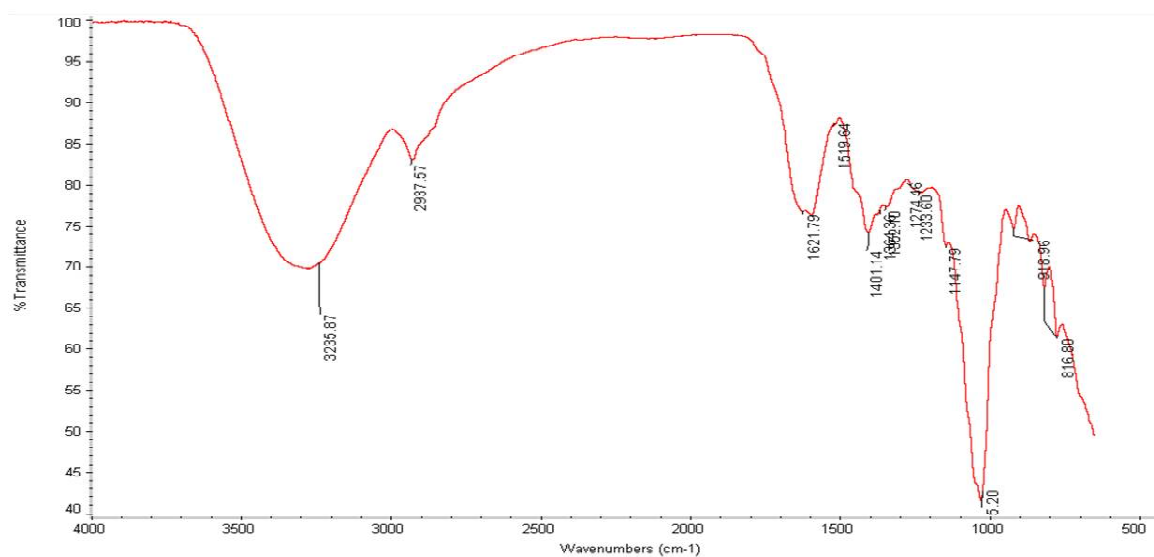


Fig. 2
FT-IR spectra of *A. mellifica*

activity (28 ± 2.84) counts in 30 minutes were observed, while in head dip test, the mice dipped head (13.33 ± 2.61) times. At the dose of 300 mg/kg the pronounced depressed effects were observed in case of light and dark, cage cross and swimming activities. Number of entries in light compartment is 9.33 ± 2.93 times. The readings of cage cross is (23.33 ± 2.44) times. In forced swimming test (FST) the Mean forced mobility time was (1.25 ± 0.04) seconds. Locomotor and exploratory activity was observed to be substantially reduced in comparison to control and standard Diazepam

(2 mg/kg^{-1}) (Farah-Saeed, 2014; Farah-Saeed *et al.*, 2015a).

Analgesic

The writhes were counted for three phases, each of 10 minutes, respectively. The inhibition of acetic acid induced writhes by aspirin was observed in all three phases: First (62%), second (21.71%) and third phase; (22.24%). *A. mellifica* at the dose of 300 mg/kg also exhibited inhibition in first (35.36%), second (21.71%) and third phases (32.36%) (Farah-Saeed 2014; Farah-Saeed *et al.* 2015b).

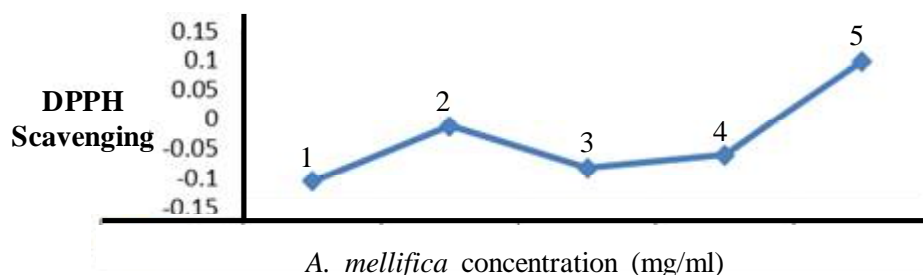


Fig. 3a

DPPH scavenging activity of *A. mellifica*

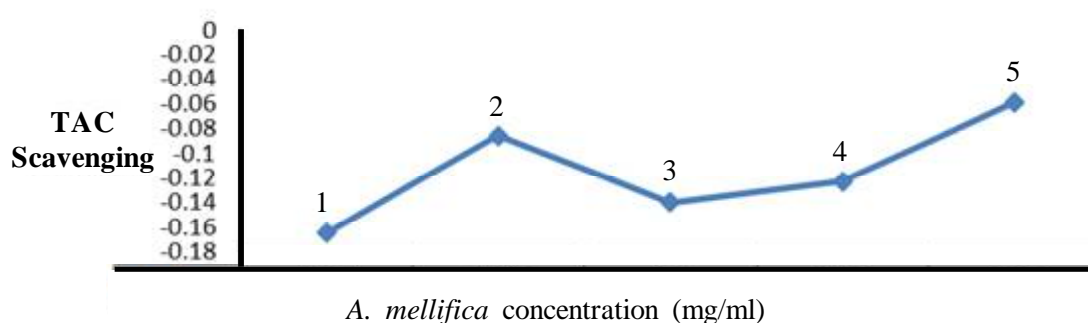


Fig. 3b

TAC activity of *A. mellifica*

Various concentration of *A. mellifica* used (mg/kg) represented as: 1, 5, 10, 50 and 100, sequentially (1-5) 2,2-diphenyl-1-picrylhydrazyl (DPPH); Total Antioxidant Capacity (TAC)

A. mellifica (500 mg/kg) showed maximum inhibition of the licking and biting response in first phase (56.60%) second phase (6.94%) and third phase (25.89%) as induced by formaldehyde (Farah-Saeed, 2014; Issam *et al.*, 2015).

Anti-inflammatory

A. mellifica (300 mg/kg) at 1.5 hours exhibited maximum paw volume inhibition 11.76%. However, *A. mellifica* (500 mg/kg) showed 25.64% maximum paw volume inhibition at 3.5 hours. Aspirin at 1.5 hours revealed maximum paw volume inhibition 22.22% (Farah-Saeed, 2014; Farah-Saeed and Ahmad, 2016b).

Kung-Woo *et al.* (2003) reported the anti-inflammatory activity of the n-hexane, ethyl acetate, and aqueous partitions from bee venom (*A. mellifera*) using cyclooxygenase (COX) activity and pro-inflammatory cytokines (TNF- α and IL-1 β) production, *in vitro*. COX-2 is involved in the production of prostaglandins that mediate pain and support the inflammatory process. The aqueous partition of bee venom showed strong dose-dependent inhibitory effects on COX-2 activity (IC₅₀ = 13.1 μ g/mL), but did not inhibit COX-1 activity (Kung-Woo *et al.*, 2003).

Anti-arthritic

Recently therapeutic efficacy of bee venom therapy in China was observed in knee osteoarthritis. In combination with conventional drugs, bee venom therapy was also found to relieve the symptoms and prevent relapse of rheumatoid arthritis (Kwon *et al.*, 2001; Liu *et al.*, 2008).

Diuretic

The mice given oral dose of 300 mg/kg of *A. mellifica* extract exhibited pronounce diuretic

activity 1.90 ± 0.0024 at the end of 4 hours as compared to the control 0.93 ± 0.0036 . Furosemide 10 mg/kg showed diuretic activity 2.52 ± 0.0033 (Farah-Saeed, 2014; Farah-Saeed *et al.*, 2015c).

Anti-urolithic

A. mellifica extracts (25%, 50%, 75% and 100%) had no inhibitory effect on calcium oxalate crystallization (Farah-Saeed, 2014; Farah-Saeed *et al.*, 2015c).

Carbon Tetrachloride Treated Rabbits

The rabbits treated with *A. mellifica* extract for three months were administered carbon tetrachloride 1.5 ml before taking out blood via cardiac puncture for LFT (Liver function test). Total bilirubin (0.033 ± 0.0046), direct bilirubin (0.023 ± 0.0046) and gamma GT (9 ± 0.632) levels were found lowered. SGPT (240 ± 0.632) and alkaline phosphatase (126.83 ± 0.658) levels were found elevated as compared to the control group (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015b).

C) Effects of *A. mellifica* on Various Parameters

Hematology

Test group (male) treated with *A. mellifica* extract, slight elevation in hemoglobin (11.75 ± 0.0836), red blood cells count (6.0 ± 0.01308), hematocrit (40.85 ± 0.0836), Mean corpuscular volume (MCV) (68.05 ± 0.0836), Mean corpuscular hemoglobin (MCH) (19.35 ± 0.0836) and total leucocyte count (7.25 ± 0.0836) was observed, while platelet count (471.5 ± 0.836) was significantly raised in test group as compared to the control group.

Test group (female) treated with *A. mellifica* extract led to a decline in hemoglobin (10.416 ± 0.0658), (RBC) Red blood

cells count (5.425 ± 0.00836), hematocrit (36.25 ± 0.0836) MCV (67.13 ± 0.1254), MCH (19.31 ± 0.0658), while an increase in white blood cells count (10.835 ± 0.0739) and platelet count (323.167 ± 0.658) was observed (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

Kidney Function

Urea (30.5 ± 0.83), creatinine (0.925 ± 0.008), serum calcium (14.45 ± 0.014), albumin (5.035 ± 0.0083), albumin/globulin ratio (A/G ratio) (2.205 ± 0.012) levels were elevated, whereas, phosphorus (4.275 ± 0.008), uric acid (0.045 ± 0.0083), total proteins (7.32 ± 0.01) and globulin (2.28 ± 0.012) were reduced in male test group treated with *A. mellifica* extract as compared to respective male control group.

Urea (40.5 ± 0.83), creatinine (0.718 ± 0.01), phosphorus (3.695 ± 0.139), total protein (7.53 ± 0.0096), albumin (4.86 ± 0.013) and A/G ratio (1.82 ± 0.009) levels were reduced, whereas, serum calcium (14.765 ± 0.0083), uric acid (0.045 ± 0.0083) and globulin (2.671 ± 0.01) levels were raised in female group treated with *A. mellifica* extract in comparison to its female control group (Farah-Saeed 2014; Mehjabeen *et al.*, 2015a).

Cardiac Enzymes

LDH (Lactate dehydrogenase) (205.5 ± 0.83) level was lowered, CPK (Creatin phosphokinase) (1758.83 ± 1.036) and CK-MB (Iso-enzymes CKM and CKB) (851.16 ± 1.036) enzymes were raised in male control group treated with *A. mellifica* extract in comparison with its respective male control group.

The cardiac enzymes; LDH (312.5 ± 0.83), CPK (842.16 ± 1.036) and CK-MB (888.67 ± 0.96) were found raised in female test group treated with *A. mellifica* extract as compare to its female control group (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

Lipid Profile

Triglycerides (287.16 ± 1.18) and Very low density lipo-protein (VLDL) (57.83 ± 1.11) were raised, however, cholesterol (40.5 ± 1.009), High density lipo-protein (HDL) (5.5 ± 0.83) and Low density lipo-protein (LDL) (3 ± 0.63) levels were lowered in male test group treated with *A. mellifica* extract as compare to its respective male control group.

Cholesterol (161.16 ± 1.036), triglycerides (45.50 ± 0.83), HDL (32.33 ± 0.96), LDL (123.5 ± 0.83), VLDL (9.42 ± 1.034) levels were raised in female test group treated with *A. mellifica* extract in comparison to its respective control group (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

Liver Enzymes

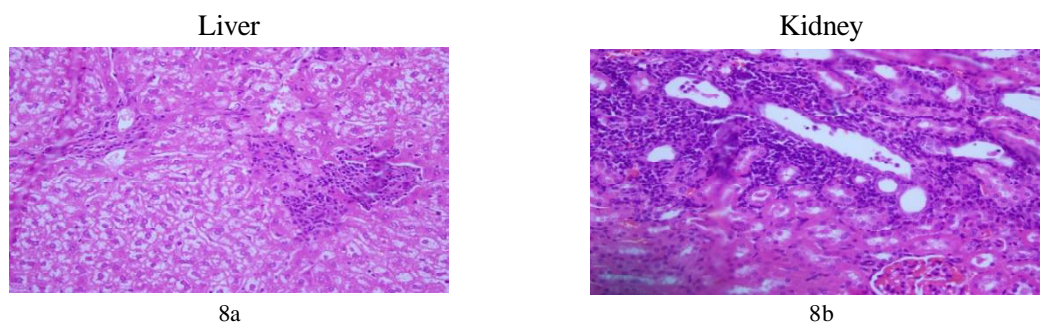
Direct bilirubin (0.12 ± 0.017) was raised, while the rest of the liver enzymes, that is, Serum glutamic-oxaloacetic transaminase (SGOT) (38.83 ± 1.036), total bilirubin (0.255 ± 0.00836), Serum glutamic-pyruvic transaminase (SGPT) (28.33 ± 1.15), alkaline phosphatase (88.5 ± 0.836) and gamma Gamma glutamyl transferase (GT) (12.5 ± 0.836) were reduced in male test group treated with *A. mellifica* extract as compared to the male control group.

Total bilirubin (0.245 ± 0.0083) was slightly reduced, while the other liver enzymes; direct bilirubin (0.065 ± 0.0083), SGOT (51.67 ± 1.154), SGPT (110.67 ± 0.96), alkaline phosphatase (101.83 ± 1.036) and gamma GT (9.5 ± 0.836) were found raised in female test group treated with *A. mellifica* extract in comparison to the respective female control group (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

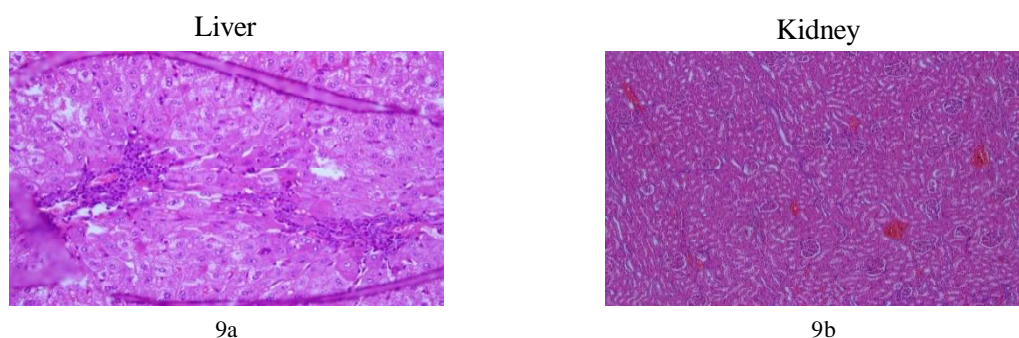
Urine Analysis of

***A. mellifica* Treated Rabbits**

The urine of the male group treated with

**Figs. 8a and b**

Histopathology of liver and kidney tissues of male rabbits treated with *A. mellifica*

**Figs. 9a and b**

Histopathology of liver and kidney tissues of female rabbits treated with *A. mellifica*

A. mellifica extract was yellow in colour and turbid, like that of its respective male control group. The pH was 9.05, slightly raised as compare to that of its respective control group. While the rest of the urine parameters of the test group were alike that of control group.

The urine parameters of the female test group were parallel to that of its respective female control group (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

Histo-pathological Studies

Old healed myocardial infarction in the wall of left ventricle and inter-ventricular septum was found in the cardiac tissues of the male

group treated with *A. mellifica* extract. No significant pathology was seen in gastric tissues. In liver tissues, mild portal inflammation and peri-portal fibrosis with foci of lobulitis was observed. Chronic nonspecific pyelonephritis was found in renal tissues (Farah-Saeed, 2014; Mehjabeen *et al.*, 2015a).

In cardiac tissues focal myocytolysis of right ventricular wall was seen, while in liver tissues, mild portal inflammation and peri-portal fibrosis with centrilobular hepatocytic degeneration was observed. No significant pathology was seen in gastric and renal tissues (Farah-Saeed 2014; Mehjabeen *et al.* 2015a).

2. CONCLUSION

A. mellifica is a very effective medicine. It is safe and effectively being used in homoeopathic system of medicine for the different organs pathologies associated with inflammation, especially of kidneys and skin.

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