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RESEARCH ARTICLE

Ameliorative role of Honey and Bee venom of *Apis mellifera* against Methotrexate Induced Alterations On Biochemical Parameters of Liver and Kidney in Male Wistar Albino Rats.

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Methotrexate, an antifolate drug is used to cure various inflammatory illness and cancer at different stages. Although it is an effective drug it has the potency to cause toxic effects on majority of the body organs. Natural antioxidants like honey and bee venom have curative properties against the side effects of the drug. The objective of the research is to examine the short-term immediate response of the drug and the role of honey and bee venom against the drug mediated changes upon the hepatic and renal function biomarkers of wistar albino rats. Rats about 10-12 weeks age and weighing around 200-250g were randomly allocated into four groups. Group 1:(Normal Control): Rats were administered 0.9% saline. Group II (Disease control): Rats were delivered intraperitoneally with methotrexate (300 µg/kg.bwt. /day) for 15 days. Group 111(Test group 1): Rats were orally administered with honey (500mg/kg.bwt/day)following bee venom(0.5mg/kg)injected intraperitoneal for 15 days. Group 1V:(Test group II):Rats were administered methotrexate 300µg/kg b.wt/day) and bee venom daily(0.5mg/kg)injected intraperitoneally with honey orally fed(500mg/kg bwt/day) through intragastric tube for about 15 days. After concluding the experiment, blood was drawn for serum separation through centrifugation at 3500 rpm for the analysis of liver function parameters like ALT, AST, ALP, Total bilirubin, albumin and kidney function indicators like creatinine, urea and uric acid. Both the organs were subjected to histopathological examination. On comparing the control group with the disease group, the administration of methotrexate to the rats (G-11) led to a significant increase in the levels of ALT, AST, ALP, Bilirubin levels whereas there was a considerable fall in the albumin level. There was also significant renal damage indicated by an increase in the levels of creatinine, urea and uric acid in the disease group. In both of the test groups, group III and group 1V the supplementation of honey plus bee venom and the supplementation of methotrexate with honey and bee venom significantly lowered the enzyme activities of ALT,AST,ALP, and bilirubin levels with an increase in the albumin level compared to disease group. Both of the test groups Group III and Group IV significantly lowered the values of creatinine, urea and uric acid than the disease group. The normal control and both of the test groups had highly significant changes when compared to the disease control group. The present study found that the combined administration of honey and bee venom of *Apis mellifera* against methotrexate mediated changes in liver and kidney function biomarkers has curative role due to its potent antioxidant, free radical scavenging and anti-oxidative activities and hence recommended against the toxic effects of the drug on different physiological aspects of the body.

Keywords; Wistar male albino rats, liver function, kidney function, methotrexate, honey, bee venom.

Introduction

Methotrexate (1989 MTX,4amino N10 methyl folic acid) was first developed in the 1940s[1,2].It is an anticancerous drug that has been shown to be highly effective against various malignancies .This drug inhibits the proliferation of malignant cells. It is widely used as a chemotherapeutic agent in the treatment of various cancer at different stages (leukaemia, lymphoma, osteosarcoma, head and

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neck tumors lung cancer, breast cancer etc) and in the treatment of various inflammatory diseases.[3].Hepatotoxicity by methotrexate is through elevation of liver enzymes, causing hepatocellular necrosis and in severe cases hepatic fibrosis and cirrhosis.[4].Liver necrosis is a result of accumulation of methotrexate polyglutamates and reduced folic acid levels[5].The limited use of the drug is due to its toxic effects involving most of the organs of the body which include bone marrow, haematological failure, liver, lung and kidney, gastrointestinal system dysfunctions[6]central nervous system[7]and gonads.[8].Drugs like doxorubicin also induce renal damage[9]. Methotrexate can elevate blood creatinine levels and lead to uraemia and haematuria. High dose causes acute renal failure.[10].The use of methotrexate lead to kidney injury evident from sharp rise in the levels of biomarkers such as creatinine ,urea ,uric acid and kim 1 along with considerable fall in the levels of albumin.[11].Renal damage is accompanied by significantly higher serum levels of urea and creatinine as well as histological changes [12].It dramatically reduce renal catalase, hepatic glutathione ,hepatic catalase activity and increase urea, creatinine, alanine, AST, ALT, ALP and renal Monodialdehyde[13].Oxidative stress is evident from low levels of superoxide dismutase, catalase, glutathione and higher tissue Monodialdehyde. The drug causes high levels of ALT,AST,TNF in the blood which is an indicator of hepatocyte injury and inflammation.[14].The histological aspects of liver and kidney is affected with the use of the drug[15-18].

Scientists have turned to natural remedies to mitigate the toxicity of drug. Emergence of complementary and alternative medicinal therapies in the form of natural is thought to be safe and effective because of their natural origin. Natural antioxidants play an immense role and aid in protection against methotrexate.[19].).In ayurvedic system of medicine various bee products have been mentioned for their therapeutic role .Natural products derived antioxidants have been reported to decrease the free radical attack on biomolecules and diminishing cumulative oxidative damage[20].Bee venom contains pharmacologically important constituents. It is formed of a complex mixture of many components including enzymes (phospholipase A2, hyaluronidase and phosphatase), polypeptides (mellitin, apamin, secapin) and low molecular compounds (histamine, dopamine, norepinephrine)[21].The most active ingredient in bee venom is mellitin that has powerful anti-inflammatory and antinociceptive effects, hence bee venom is used to treat inflammatory diseases[22].Mellitin has the potency to supress cisplatin induced increase in renal biomarkers like creatinine and Blood Urea Nitrogen. [23].Recent studies reported that bee venom possess antioxidant activity [24],antimutagenic [25],proinflammatory[26], anti-inflammatory[27],, antinociceptive [28], and anticancer effects [29].Bee venom also has a radioprotective effect against basal and oxidative DNA damage in wistar rat lymphocytes[30]. Phospholipase A2 in the bee venom has significant anti-inflammatory and arthritic properties.[31]. Through downregulating proinflammatory cytokines and chemokines bee venom reduces pain and edema in gouty arthritis rats [32]. In a phase III clinical trial administration of bee venom ,it sustainedly improved knee osteoarthritis pain and body function [33].
Honey is also considered a significant source of antioxidants because of its rich in phenolic acids, flavonoids, ascorbic acid and carotenoids [34]. Honey has protective effect against the damage to liver and kidney induced by cisplatin [35]. The antioxidant properties of honey cure oxidative damage to organs including kidney, pancreas, liver and heart[36]. Manuka and talh honey has ameliorative properties against cisplatin induced hepatotoxicity and nephrotoxicity[37]..The administration of honey improved the function of kidney than the cisplatin receiving group[38].Therefore combining use of honey and bee venom with methotrexate might reduce the side effects induced by the drug .More experimental studies need to emerge regarding the natural products like honey and bee venom .In the present study honey and bee venom is combined with the drug to investigate its curative role against drug toxicity upon liver and kidney.

MATERIALS AND METHODS

Animals

Healthy male wistar albino rats weighing 200-250g of about 10-12 weeks age were obtained from the animal house of Crystal Biologicals, Pune. All the experimental procedures such as Housing, Dosing, Sacrifice, rehabilitation was done in accordance with the standard operating procedure and guidelines provided by the CPCSEA published in the Gazette of India, Dec 15,1998 and Biological evaluation of medical devices-Part 2: Animal welfare requirements. The study was approved by IAEC meeting of Crystal Biological Solutions, Pune (Reg No-2030/PO/RcBiBt/S/18/CPCSEA)

Housing Conditions

The rats were housed in polypropylene cages and the bedding material was clean paddy husk which was changed every day. Room temperature was maintained between 22±3°C, relative humidity 55±5% and 12 hours light dark cycle was maintained. Identification marks were given to cages and animals. Maximum three rats were placed in each cage and

stainless-steel cage tops with facilities for food and water were provided.

Acclimatization
The rats were categorised into four groups with 6 rats in each group and they were acclimatized at test environment for seven days prior to experimentation. During this period the animals were observed daily for clinical signs.

Diet

Commercial pelleted feed supplied by Nutrivet Pvt Ltd ad libitum

Water
Portable water passed through RO unit provided ad libitum in polypropylene bottles with stainless steel sipper tubes.

Chemicals
Methotrexate was purchased from Ipca Laboratories Ltd. Bee Honey and Bee venom was collected directly from the *Apis mellifera* colonies located in the University Campus. Food pellets were purchased from Nutrivet Pvt.Ltd, Pune. SGPT, SGOT and ALP was purchased from SPINREACT. Albumin from Delta Lab and Bilirubin from Pathozyme. Urea from Care one, Uric acid from AGD Clinipak and Creatinine from delta lab.

Preparation of bee venom and honey
Bee venom. Honeybee venom was obtained based on electric shock method. Dosage Bee venom was freshly prepared by dissolving in distilled water just before treatment, and was administered every day. Bee venom was injected intraperitoneal (IP) with 0.5 mg/kg dose.
Honey- 500mg of honey was dissolved in distilled water and administered through an intragastric tube through the mouth of rats. The doses were weighed on digital scales where each dose relied on the relevant animal's weight.

Experimental Design
Inspired from the study [39] the protocol was systematically remodified and the present study focused on intraperitoneal administration of the drug at 300µg dose for a duration of 15 days. The rationale behind selecting the intraperitoneal route was particularly to facilitate rapid drug delivery and onset of action and the time duration was confined to 15 days to specifically examine the immediate and short term response to the intervention thus examining specific endpoints like biomarker changes in the initial phase of treatment.The study aimed to explore the potential ameliorative role of bee venom against methotrexate induced effects on rat.The study hypothesized that bee venoms anti-inflammatory and antioxidative properties aid protection against methotrexate and bee venom was employed at 0.5mg/kg from its efficacy and the administration of 0.5mg /kg bee venom was set up on the basis of standardised evaluation of bee venom in different experimental paradigms. [40,]. Through adjusting the dosage of honey to 500mg in the study, its efficacy was studied against methotrexate toxicity.[41,42].
For the study,24 adult male wistar albino rats weighing 200 - 250 and 10 - 12 weeks age was randomly divided into four groups each group containing 6 rats(n=6) and were treated for 15 days as given below:
Group 1(Normal Control):0.9%(10ml/kg/day) saline solution was administered for 15 days.
Group2(Disease Control): Methotrexate (300µg/kg.bwt/day)was intraperitoneally injected for 15 days
Group 3(Test group 1-Honey and Bee venom): Honey(500mg/kg/day) orally fed + Bee venom(0.5mg/kg.bwt/day) intraperitoneally administered for 15 days.
Group 4(Test group 2-Methotrexate + Honey + Bee Venom):300µg/kg/day of methotrexate injected intraperitoneally while honey (500mg/kg/day) orally fed and Bee venom(0.5mg/kg/day) injected intraperitoneal for 15 days.

Blood Collection and analysis of Serum sample
After 15 days of treatment, blood was drawn from the retro orbital plexus under light ether anaesthesia from all the groups. The collected blood was placed in tubes devoid of anticoagulant and the serum was separated through centrifugation at 3500 rpm at 25C for 10 mins. The biochemical parameters including SGOT, SGPT, ALP, bilirubin, albumin, creatinine, urea and uric acid were estimated by using commercially available kits. The entire biochemical estimation was performed at Crystal Biologicals, Pune.

Histopathological studies
Animals were anesthetized using Carbon dioxide and chloroform and sacrificed by cervical dislocation. The liver and kidney were dissected out carefully and excised and fixed in 10% formalin and was hydrated in ascending grades of ethanol, cleaned in xylene and embedded in paraffin. Sections were cut and performed under light microscope in terms of changes in different groups with respect to the disease group. Results obtained is presented in microphotographs.

Statistical Analysis
The present study for optimization curative effects of honey and bee venom was analysed by applying one-way anova and 2-way anova by Dunnett's multiplying

comparison test and accomplished using Graph Pad Prism 9 software. The result was confirmed to be significant with P value less than 0.05 (P≤0.05)

RESULTS

Results of Liver function

The control group, the honey and bee venom group, methotrexate with honey and bee venom - group were evaluated in comparison with the methotrexate group for the analysis of liver biochemical parameters and the result obtained is summarised in table no 1.

The treatment with methotrexate significantly increased levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)Alkaline phosphatase, Bilirubin compared with control on the other hand there was a slight decrease in the albumin level compared to control.

The treatment with Test group 1(Honey and bee venom group) and test group 2(Methotrexate with honey and bee venom) led to significant decrease in the activity of ALT, AST ALP, and bilirubin compared to the methotrexate administered group and the serum level of albumin significantly increased in the test group 1 and in the test group 2 than the drug administered group.

TABLE 1

Groups	Albumin (g/dl)	SGPT (U/L)	
Normal Control	4.26±0.25**	48.91±3.03****	
Disease control	3.59±0.30	117.77±4.77	
Test 1	4.14±0.33**	56.98±4.51****	
Test 2	4.27±0.22**	53.16±7.22****	
Groups	**SGOT (U/L)**	**ALP (U/L)**	**Total Bilirubin (mg/dl)**
Normal Control	88.35±3.79****	87.88±3.68****	0.53±0.04****
Disease control	265.41±23.77	381.49±7.55	1.25±0.04
Test 1	111.31±6.47****	96.29±1.72****	0.82±0.03****
Test 2	95.43±7.81****	89.50±1.45****	0.69±0.08****

Values are mean ± SD, n = 6 in each group.
P ≥ 0.05 non- significant
***P <0.05 when compared with Disease Control**
****P <0.01 when compared with Disease Control**
*****P <0.001 when compared with Disease Control**
******P <0.0001 when compared with Disease Control**

Normal Control and Test group animals showed significantly lower values of SGPT, SGOT, ALP, and Bilirubin and slightly increased albumin. Highly significant changes were obtained in Normal control and Test group animals ($p<0.0001$) when compared to the disease control group.

Disease control animals showed significantly increased SGPT, SGOT, ALP, Bilirubin and decreased albumin after treatment of methotrexate drugs.

One way anova was used to find out difference between the groups.

Results of Kidney Function

The control group, the honey and bee venom group, methotrexate with honey and bee venom group were evaluated in comparison with the methotrexate group for the analysis of kidney function biomarkers and the result obtained is summarised in table no 2.

The treatment with methotrexate significantly increased levels of creatinine, urea and uric acid compared with control. The treatment with test group 1(Honey and bee venom) and test group 2 (Methotrexate with honey and bee venom) led to substantial decrease in the levels of creatinine, urea and uric acid in comparison to the drug administered group.

TABLE 2

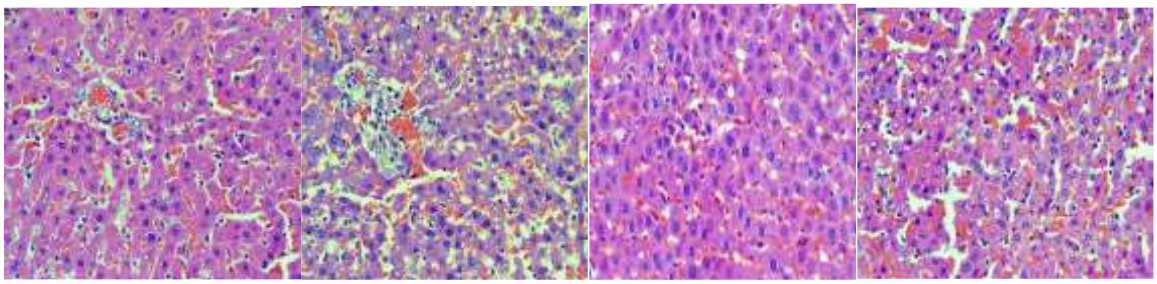
Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl)
Normal Control	0.68±0.02****	16.75±1.08****	6.04±0.33****
Disease control	0.91±0.02	33.79±2.82	8.33±0.30
Test 1	0.72±0.01****	19.13±0.54****	6.66±0.48****
Test 2	0.69±0.01****	17.87±0.64****	6.27±0.16****

Values are mean ± SD, n = 6 in each group.
P ≥ 0.05 non- significant
***P <0.05 when compared with Disease Control**
****P <0.01 when compared with Disease Control**

*****P <0.001 when compared with Disease Control**
******P <0.0001 when compared with Disease Control**

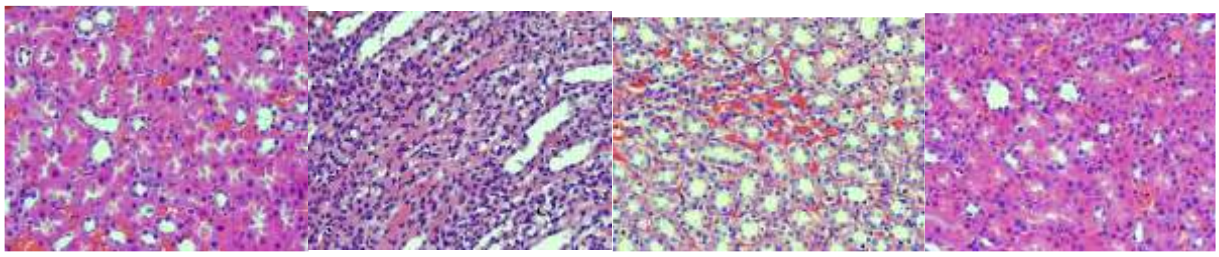
Normal Control and Test group animals showed significantly lower values of creatinine, urea and uric acid. Highly significant changes were obtained in Normal control and Test group animals1 ($p<0.0001$) when compared to the disease control group.
Disease control animals showed significantly increased creatinine, urea and uric acid values after treatment of methotrexate drugs.
One-way anova was used to find out difference between the groups.

Histopathological Observation of Liver
The normal control group showed normal centrilobular area and hepatic parenchyma. In the disease control, the drug administration resulted in hepatocellular vacuolation, degeneration, necrosis with infiltration of inflammatory cells. Both the test groups I and II reversed the damage caused due to methotrexate and normalised the liver histology through normal centrilobular area and hepatic parenchyma. The histopathology of one of the rats from each of the four groups is given here.



Group 1 Group 2 Group 3 Group 4

Histopathological observation of kidney
Normal renal tubules in cortex and medulla were found in the control group. In the disease control there was clear infiltration of inflammatory cells in the cortex and medulla. Both test groups I and II had normal renal tubules in cortex and medulla as the administration of honey and bee venom and honey and bee venom with drug reversed the damage. The histopathology of one of the rats from each of the four groups is given here.



Group 1 Group 2 Group 3 Group 4

Discussion
The derived findings suggests that the methotrexate induction in rats caused significant increase in the Aspartate aminotransferase, Alanine aminotransferase, Alkaline phosphatase, Total bilirubin. On the other hand the serum albumin was slightly decreased compared to control. Similar findings were reported by previous investigators[43,44].The elevation in the transaminase level of the liver is due to the retention and accumulation of methotrexate polyglutamates [45,46]. MTX-polyglutamate ,a metabolite of methotrexate and its accumulation is associated with oxidative damage, inflammation, hepatic lipid accumulation , fibrosis, and cell death in liver cells. The metabolite has the potency to cause oxidative injury in the liver by causing lipid peroxidation resulting in discharge of reactive oxygen species and downregulating antioxidant response elements[47].The result of the study corroborates with the investigation on the effect of polyherbal formulation on methotrexate induced hepatotoxicity in rats[48].Liver impairment is thought to be the reason for elevated serum AST and ALT[49].The present study with elevated enzyme level is due to hepatic damage which release the enzymes into bloodstream and this hepatic damage can influence structure and functional stability of cell membrane associated with several hepatic diseases[50].The study findings match[14,13,51,52] who examined that, the administration of methotrexate resulted in significant increase of serum ALT and AST levels. The oxygen radicals and hydrogen peroxides , the free radicals cause cell damage after adhering to cellular polymers, in particular membrane lipids leading to discharge of ALT and AST from cells to

serum[53].Hepatotoxicity induced by methotrexate is via its interaction with dihydrofolic reductase which inhibits conversion from folic acid to folinic acid that impede nucleic acid synthesis ,amino acid and proteins indirectly. Organelles and hepatic plasma membrane is damaged thus favouring enzyme discharge[2,54-58].The increased toxicity of methotrexate and its removal at a slower rate is thought to be the reason for lowered albumin level. Albumin is a serum protein to which methotrexate binds —and reduced indicate extended drug elimination and hepatic dysfuncton[59,60].There were significant higher levles of ALP activity with methotrexate administration found parallel to previous studies[52,61,13,62].Elevated ALP is a marker of liver damage,indicating hepatotoxicity which discharge the enzyme into the bloodstream[52].An increase in bilirubin level was found parallel to Mahmoud et al., 2017 and attributed to increased production of free radicals[64].

The honey and bee venom decreased the level of liver function parameters than the disease control group and renewed the levels of AST, ALT, ALP and bilirubin . The liver damage and lipid peroxidation induced by LPS and carbon tetrachloride was cured through curative properties of honey and Mellitin and Mellitin works against liver damage in rats by isoniazid- and rifampicin revealing the combined administration of of BV and honey, or Mellitin prevents liver damage and can used as potential therapeutic agents[65,66].Melittin in bee honey cure acute liver damage[67]. Phospholipase A2 significantly cure cholestatic liver injury in mice via the obstruction of inflammation and liver cell apoptosis [68].

Honey reduces ALT and AST levels with its bioactive compounds such as kaempherol, quercetin, chrysin, luteolin, apigenin, and vanillic acid and these compounds reduces ALT and AST through maintenance of biomembrane integrity, lowering free radical species, and regulation of liver metabolism. The treatment with bee venom had significantly decreased the elevation of serum ALT, AST levels in rats exposed to gammaradiation (5 Grays) indicating the hepato protective effect[69,70] .Bee venom . suppress oxidative stress, tubular cell apoptosis and inflammation of an acute kidney [23].

Administration of methotrexate to rats in the disease group resulted in a significant increase of creatinine, urea and uric acid compared to control and both the test groups 1 and 2.The result corroborate with previous investigations. Methotrexate noticeably lowered renal catalase, liver glutathione and liver function while considerably elevating levels of urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and renal malondialdehyde whereby rosmaniric acid reduced AST and ALT values [13].The renal injury is always associated with elevated uric acid levels and increased uric acid level is associated with different detrimental pathological and cellular phenomenon like reactive oxygen species production and dysfunction of endothelium[[71].The drug resulted in the renal damage marked by significantly higher levels of serum urea and creatinine accompanied by histological changes. [12].The kidney function, biomarkers of oxidative stress, histological, and immunohistochemical changes in methotrexate group significantly outperformed the control group in terms of blood urea nitrogen, creatinine, uric acid, tissue oxidative stress markers, total oxidant status, oxidative stress index levels, and total antioxidant status levels.[72].As nucleotides metabolize ,uric acid is produced and eliminated via kidneys[73,74].Urine pH less than 7 due to high methotrexate dose is a marker of nephrotoxicity [75].The delay in the clearance of high level methotrexate contributes to dysfunction of the kidney and associated rise in renal markers[76].Crystal nephropathy induced by high dose methotrexate causes kidney injury due to deposition of drug and associated metabolites in the tubules of the kidney. Drug being acidic in nature cannot be present in urine having basic nature since increased pH of the urine enhance the solubility and the excretion of the drug. Crystal-mediated renal injury results in asymptomatic rise in serum creatinine and further leads to tubular necrosis and more severe renal injury[77]..The drug has the potency to cause kidney reactive oxygen species ,lipid peroxidation, glutathione reduction, decline in total antioxidant capacity and the histological examination recorded interstitial nephritis,, necrosis of renal tubules, retraction glomeruli, and vascular congestion . The mitochondrial indicators like the mitochondrial membrane potential, performance of mitochondrial dehydrogenases , and mitochondrial glutathione and ATP reduced on the other hand lipid peroxidation and mitochondrial permeabilization increased upon methotrexate administration indicating the significance of mitochondrial dysfunction and oxidative stress in methotrexate mediated toxicity to kidney[78].A single dose of methotrexate resulted in improper functioning of the renal system marked by elevated serum urea and creatinine levels with high urine albumin/creatinine ratio along with significant decrease in the serum albumin[79].The increased production of ROS in nephrons adversely affect nephrons bringing out morphological and physiological alterations of renal tubules and glomeruli [80].Uric acid is produced during the metabolism of nucleotides and excreted mainly by the kidney [73,74].The generation of reactive oxygen species from oxidative injury and reactive nitrogen species results in kidney inflammatory cascade and

damage of cells , along with oxidation of biological molecules consisting lipids, proteins, and DNA, causing renal damage[81,82-83].
The treatment with honey and bee venom reduced the toxicity and the results were parallel with previous investigations. The damage to kidney is reversed through administration of honey, royal jelly and propolis[9]. Honey and bee venom has curative properties on the functional indicators of the renal system. Lysozyme-like and phenolic acids, i.e., coumaric, caffeic, cinnamic and syringic acids in honey have profound role in curing health. [84].Honey constitutes caffeic acid , caffeic acid phenyl esters, Chrysin , Galangin , Quercetin , Kaempferol , Acacetin , Pinocembrin , Pinobanksin , and Apigenin, effective substances in chemotherapy[85].The LPS-induced acute renal damage was cured by Bee venom, Mellitin, and Apamin through inhibition of free radical damage, inflammation and cell death in mice [86,87,68].Honey and propolis work against carbon tetrachloride hepatotoxicity in rats[88]..Honey prevent amikacin induced nephrotoxicity in rats[42]. . Bee venom has anti-oxidants, anti-coagulants, anti-inflammatory properties and biologically active compounds like mellitin and phospholipase A2 that work against inflammatory disorders[89].Propolis associated with bee venom considerably reduced the elevated values of blood urea and creatinine in the irradiated group approaching control[90].) Propolis and/or bee venom treated rats significantly had lower levels of total protein, albumin, alanine aminotransferase, aspartate aminotransferase, and Alkaline phosphatase in comparison to control irradiation rats. Crude bee honey and royal jelly capsules lowered serum levels of renal injury products (creatinine and urea0[91]. Bee venom and propolis + bee venom significantly decreased blood levels of urea and creatinine [92].The honeys antioxidant, anti-inflammatory and antimicrobial property with its potency to combat oxidative injury and inflammation promotes liver and kidney health[93].The antioxidant mechanism of honey and bee venom reduces oxidative stress through scavenging free radicals which in turn leads to reduced enzyme levels of hepatic and renal system.[94]. Bee venom administered to adenine-treated mice improved renal function altering physiological and biochemical indices, bee venom is a curative remedial agent to improve renal function in adenine-administered chronic kidney disease. [95]. Histopathological analysis of liver showed normal architecture in the control group but the disease group had hepatocellular vacuolation ,degeneration, necrosis with infiltration of inflammatory cells. Both the test groups I and II reversed the damage caused due to methotrexate and normalised the liver histology through normal centrilobular area and hepatic parenchyma. The findings were concurrent with previous investigations[96,97,98.99].There was severe fatty alteration , hepatocyte degeneration , clogged portal tract , missing cell borders, and deformation of normal architecture .The hepatocytes showed hyperplasia of fibrocytes and infiltration of mononuclear white blood cells, coagulative necrosis and hepatocellular vacuolation[100]. The drug causes inflammation, hepatocyte vacuolization, dilatation, and congestion associated with severe hepatocyte vacuolization, sinusoidal dilatation, radial organisation disruption, and severe congestion in the stromal areas [101].Mild vasculitis and degenerative vacuolation of the hepatocyte is reflected in most of the hepatic sections[14].
The ultrastructure of hepatocyte is improved by the combined treatment of CdCl2 and honey[102].The nonprotein sulfhydryl groups in few amino acids and honey like anzer honey ameliorated toxin-induced morphological changes[103].Honey plays an ameliorative role to shield rat liver and kidney cells from oxidative damage[104]. The liver histological abnormalities, renal damages, altered lipid profile, haematological failures induced by IM(imidacloprid) were reduced in rats fed with Sider honey and low dose of zinc indicating the antioxidant qualities of Sider honey[105]..Bee venom prevents liver fibrosis[67].

Renal and hepatic toxicities associated with concurrent methotrexate therapy leads to haemorrhagic diathesis and reduced survival[106]. Renal cortex, atrophied glomeruli, lobulated glomeruli, capsule space, proximal and distal convoluted tubules, and renal capsule with sloughed collagen fibre was found in the methotrexate-treated group. [107]. Methotrexate inhibits the dihydrofolic reductase enzyme, which has a direct harmful effect on the renal tubular cells [108]. Acute kidney injury (AKI) resulting in severe renal damage was found and upon further assessment ANCA-associated small-vessel vasculitis with rapidly progressive glomerulonephritis (RPGN) was reported. [109].The normal structure was impaired with degeneration of the glomeruli and congestion. There were affected renal tubular cells, haemorrhage in the interstitial space and inflammatory infiltration associated with significant increase in collagen fibres and decreased PAS staining. Ultrastructure studies marked mitochondrial degeneration, cytoplasmic vacuolation of cytoplasm with basement membrane damage in kidney tubules[110].The venom has positive role due to its anti-tumour ,neuroprotective, anti-inflammatory ,analgesic ,anti-infectivity effect[111].The microscopical anatomical structure of kidney is prevented and the damage to it is mitigated through natural products like bee venom from *Apis mellifera intermissa*[95].The histological

injury caused to Unilateral Ureteral Obstruction mice was reversed trough bee venom administration[112].

CONCLUSION

Honey and bee venom restored liver and kidney function parameters through their ameliorative properties against hepatic and renal damage. It is concluded that administration of both honey and bee venom has a protective role against changes induce upon liver and kidney of rats by methotrexate. The anti-inflammatory and anti-oxidant characteristics of both honey and bee venom works against hepatotoxicity and nephrotoxicity.

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