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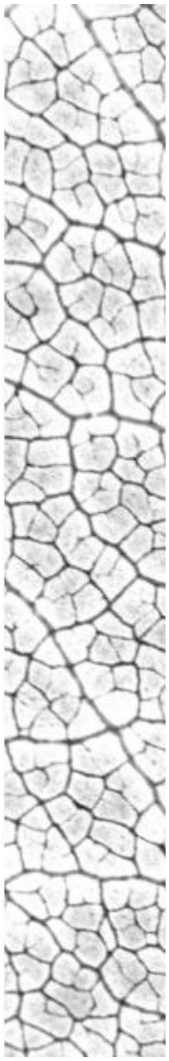

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Garlic extract and honey as potential protective agents against cadmium-induced Nephrotoxicity in male rats

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Abstract

Background: Cadmium (Cd) contamination is a very serious international problem as it can enter into the food chain, become bio-accumulated, and endanger human health. **Aim**: To determine the protective aqueous potentials of the extract of garlic and honey on toxicity induced by cadmium (Cd) at rats. **Materials and Methods:** Rats (35 males) were haphazardly categorized into seven groups. Standard group (1) received D.W.; group (2) received aqueous (GAR) extract (two g/kg BW); group (3) received honey (HON) (1,5 g/kg BW); group (4) received CdCl2 (5 mg/ kg BW); Group (5) received aqueous (GAR) extract plus(HON); Group (6) received aqueous (GAR) extract plus (HON), after 6 hour received CdCl2; group (7) received CdCl2 for 4 weeks and then treated with aqueous (GAR) extract plus(HON) for additional 4 weeks. Doses being tested were given orally to rats by gavages approximately for 8 weeks (3 times a week). **Results:** showed a significant increase in serum urea, creatinine, uric acid, and MDA while a significant decrease in SOD, GSH, and CAT in CdCl2-intoxicated animals (group 4) compared to the control group. Treatment of CdCl2-intoxicated animals with aqueous garlic extract and honey revealed a decline in the harmful Cd influences via restoring antioxidant activities, biochemical modifications, and oxidative stress markers.

Keywords: cadmium, nephrotoxicity, garlic, honey, antioxidant

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INTRODUCTION

Cadmium (Cd) is a ubiquitous environmental pollutant (Boonpeng *et al,* 2016), it is intensified in the chain of food because of its nature being highly soluble in comparison to other heavy toxic metals in which it is not degradable; and as a sequence, it is transported easily to plants from the soil where humans and animals highly depend on it for surviving (Oyinloye *et al,* 2016). Cadmium was used widely in industry being agent of anticorrosive for brass, iron, steel, and different alloys, and as paints stabilizer, pigments, plastics, and batteries of nickel-Cd (M Brzóska *et al,* 2016).

Cadmium accumulates mainly in the liver and kidney with extreme long half-life biologically in humans (10-30 years) renders it as toxin being cumulative. It is well known that chronic Cd exposure able to induce nephropathy severely in animals and humans (Sadek *et al,* 2017; M Brzóska *et al,* 2016). Injury of renal is thought to be attributed to metallothionein of Cd that is produced originally at liver, liberated into circulation, absorbed via epithelial cells located at proximal renal tubular, and degenerated to Cadmium ions being liberated as toxic (Boonpeng *et al,* 2016).

The health risk damage via Cadmium at conditions of environment (Oyinloye *et al,* 2016) along forecasts where population in general are exposed Cadmium will elevate in future (Ferraro *et al,* 2010). Hence, more researchers interests has been concentrated on finding powerful population protection ways from harmful exposure effects to Cadmium along poisonings treatment with Cadmium. Thus, the effective poisonings treatment of Cadmium should be able to remove it from cells and body elimination (Wallin *et al,* 2014). Also, to block absorption of Cd into body of organism and its body detention together increasing the organism ability defending against heavy Cadmium toxicity. Various nutrients and medical plants might prevent absorption of Cd from the GT and some influences of Cd toxic action. Due to various damaging effects of Cadmium action resulting from its properties being pro-oxidative strongly (Galażyn-Sidorczuk *et al,* 2012).

Vegetables and fruits consumption was reasoned to advantageous health influences. Generally, vegetables and fruits have non-nutrients and nutrients which modulate and control different body functions in which contributing to a steady health state maintenance and minimize diseases risk. Vegetables and fruits are enriched with phytochemicals (chemicals being active biologically along nutrients) which assist in diseases prevention and becoming a major interest to public health and scientific community (Prior, 2003). Garlic is outstanding among the health-promoting vegetables. It is widely and versatile

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accepted by most cultures. Garlic is enriched in compounds of organosulphur of metal-chelating antioxidant and characters together with modulating systems of detoxification and inflammation (Boonpeng *et al,* 2016). Garlic's components have functional influences of benefits which include: Anti-carcinogenic effect, anti-diabetic effect, antiasthmatic effect, anti-osteoporotic effect,anti-cataractogenic effect, hypocholesterolaemic effect (Eteng *et al,* 2012).

Honey is viscous and sweet fluid synthesized via honeybees and derived from flowers nectar. Honey have many minerals and vitamins at trace amounts (Atagana & Asagba, 2014). Recently, there is a high interest in antioxidants application as medical treatment where data is available connecting human diseases development to OS (Aliyu *et al,* 2012). Honey considered as antioxidant being natural having ascorbic acid, flavonoids, catalase (CAT), tocopherols, and phenolic compounds providing a synergistic antioxidant influence, free radicals eliminating and scavenging (Erejuwa *et al,* 2012; Abdelaziz *et al,* 2013).

The main objective of the proposed study was to examine the possibility of using garlic (GAR) extract with honey (HON) as a protection or treatment against cadmium toxicity (Cd) in kidneys of test animals and assessing the antioxidant activity of aqueous extract, using several antioxidant assay systems. Also, examine the potential of aqueous extract in removing cadmium toxicity from the kidney tissues and blood of tested animals.

MATERIALS AND METHODS

Chemicals

All reagents were of the highest quality. Reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB), nicotinamide adenine dinucleotide phosphate (NADPH), trichloroacetic acid (TCA) and most other chemicals were purchased from Sigma Chemical Company (Saint Louis, USA). All kits were purchased from Biodiagnostic, Egypt.

Tested compounds and doses

Cadmium Chloride ($CdCl_2$) was purchased from Sigma Chemical Company (Saint Louis, USA). $CdCl_2$ was prepared by dissolved in distilled water, the dose of 5 mg / kg of body weight was chosen according to (AL-Shemmary, 2005) study.

Tested aqueous extract

Garlic (GAR) and Bee honey (HON) were obtained from a known source in the local market. Aqueous garlic extract, was prepared by drying the garlic and cut it into small pieces, weighing 50 grams, put it in a glass bowl, add 250 ml of distilled water to it and crush it with an electric mixer for a very short period. The extract is obtained by separating the juice using filter paper, the garlic aqueous extract is placed in sterile dark glass containers with a tight cover and in conditions without moisture and kept in the refrigerator until used in the study (Kawthar, 2007). The honey solution was prepared by dissolving honey in distilled water (25% W/V), the dose of 1.5 mg / kg of body weight was chosen according to (Veronika *et al*., 2004) study.

Animal grouping and dose administration

Thirty five healthy adult male rats (Sprague Dawely) with average weight of 155~165g, 12~14 weeks age, were obtained from the faculty of NATIONAL CENTER FOR DRUG CONTROL AND RESEARCH in Baghdad. Rats were housed in the animal house in the biology department / College of Education for Pure Sciences / Anbar University, in cages maintained at 20–25°C with 40–70% relative humidity. Animals had *ad libitum* access to a certified rodent basal diet, and sterilized tap water was given through water bottles, taken in consideration that water, body weights (loss or gain) and consumption of food were weekly registered till the experiment termination. All rats had no previous abnormal clinical conditions before the study. After 2 weeks of acclimation; rats were haphazardly classified into 7 equal groups. Standard group (1) received D.W.; group (2) received aqueous (GAR) extract (2 g/kg BW); group (3) received honey (HON) (1,5 g/kg BW); group (4) received $CdCl_2$ (5 mg/ kg BW); Group (5) received aqueous (GAR) extract plus (HON); Group (6) received aqueous (GAR) extract plus (HON), after 3 hour received CdCl2; group (7) received CdCl2 for 4 weeks and then treated with aqueous (GAR) extract plus(HON) for additional 4 weeks. Doses being tested were given orally to rats by gavages approximately for 8 weeks (3 time a week).

Collection of blood samples and isolation of kidney tissue

At the experiment, all rats were under anesthetize with diethyl ether and sacrificed then collection to samples of blood were performed from the heart Vena Cava in heparin collecting tubes. For separation of plasma, centrifugation was done for blood at 860 xg for twenty min. and at - 80°C; plasma was kept till tested parameters analyses. Kidneys were isolated, weighed, then with saline washed and kept in formalin (3%) for for histological studies.

Kidney functions

Urea and Creatinine were evaluated based on Patton & Crouch and Schirmeister methods, respectively. UA was measured according to Barham & Trinder method.

Markers of oxidative stress and parameters of antioxidant

Malondialdehyde (MDA) was evaluated according to the method of Ohkawa. Superoxide dismutase (SOD) activity was assayed by the method of Nishikimi, also Glutathione (GSH) level was assayed regarding Beutler method. CAT was measured according to the described method of (Iwase et al. 2013). The above-mentioned parameters were measured in compliance with Biodiagnostic Kit, Egypt manual instruction.

Examination as histopathological

Rats' kidney tissues were cut and fixed immediately into solution of 10% formalin and dehydrated utilizing alcohol and xylene grades ascendingly. Tissues were put in xylene and molten wax for almost ten min. then in paraffin wax were embedded and finally via Rotary microtome were sectioned for obtaining sections at 4–6 µm thickness. Following that staining was done by hematoxylin and eosin

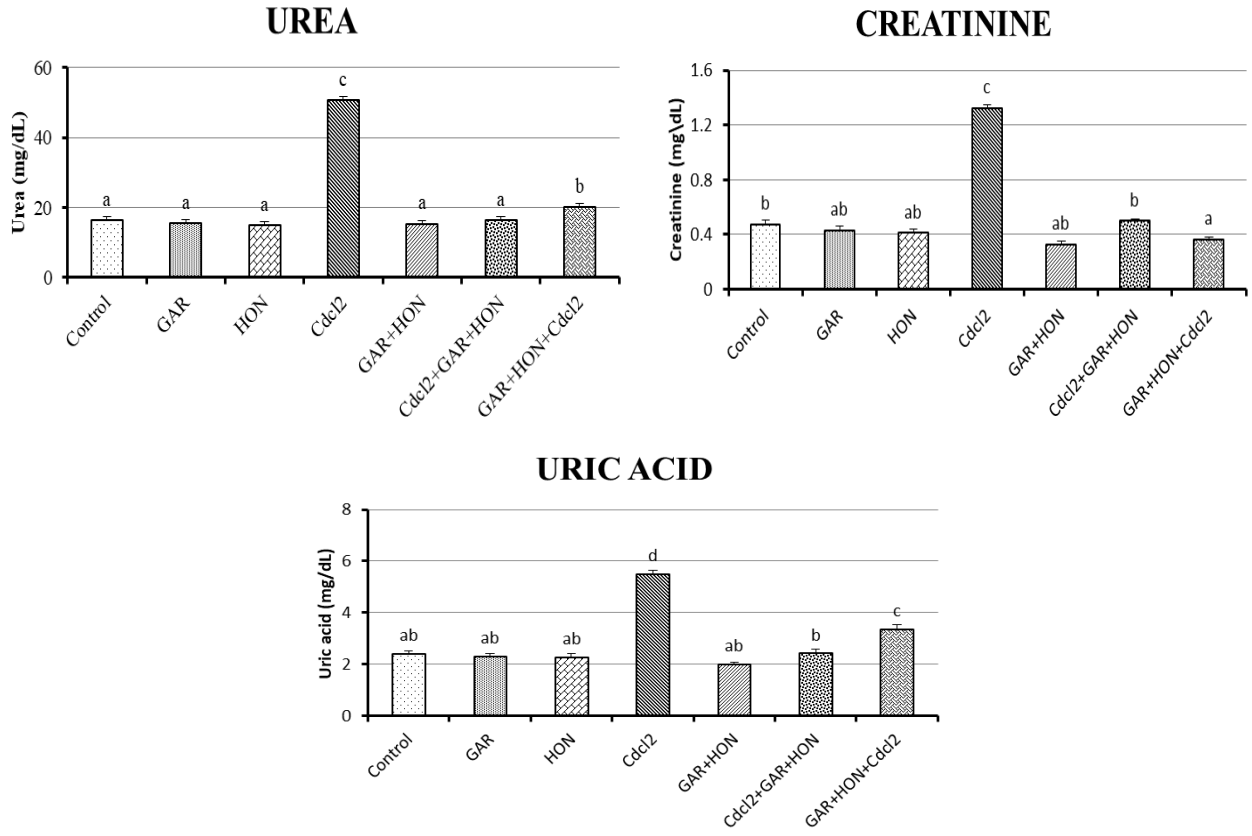

Fig. 1. Effects of cadmium and protective role of GAR + HON in Urea, Creatinine and Uric acid in blood serum of male rats. The results expressed as (Mean ± SE)
abcd = values at column with small different letters are different significantly at (P<0.05)

to investigate changes as histopathological based on method of Drury & Wallington.

Statistical analysis and statistics

Values of mean and standard error were detected for all measured parameters where results were exposed as Mean ± SE. Data was analyzed using a one-way (ANOVA) then by Duncan multiple comparisons. P<0.05 was statically significant according to Norušis method**.**

RESULTS

The animals under oxidative stress by CdCl2 in group 4 showed a significant (0.05≥P) increased in Urea, Creatinine and Uric acid compared with the control group. Meanwhile it showed significantly (0.05≥P) decreased in group 6 and 7 when compared to the group 4 (CdCl2 group). Animal dosing with aqueous extract of (GAR+ HON) in groups 6 was able to approximate levels of urea, creatinine and uric acid with the level of the control group, while the level of urea and uric acid was still higher than those of the control group but creatinine was lower than those of the control group despite the stressed animals dosed with the aqueous extract for 30 days as shown in **Fig. 1**.

Results of oxidative stress markers in **Fig. 2** showed that MDA significantly (0.05≥P) increased in groups 4 (CdCl2 group) when compared with control group, while groups 6 and 7 that treated with (GAR+HON) aqueous

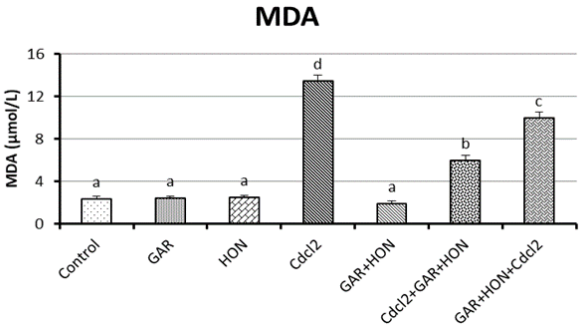

Fig. 2. Effects of cadmium and protective role of GAR + HON in MDA in blood serum of male rats >The results expressed as (Mean ± SE)
abcd = values at column with small different letters are different significantly at (P<0.05)

extract significantly (0.05≥P) decreased when compared with groups 4 (CdCl2 group) but showed a significantly increased when compared with control group.

As shown in **Fig. 3**, the activities of antioxidant enzymes SOD, GSH and CAT have been significantly (P < 0.05) declined in the group 4 ($CdCl_2$ group) when compared with the control group. The presence of aqueous extract of (GAR +HON) in the group 6 and 7 was capable to elevation the activities of these enzymes compared with group 4

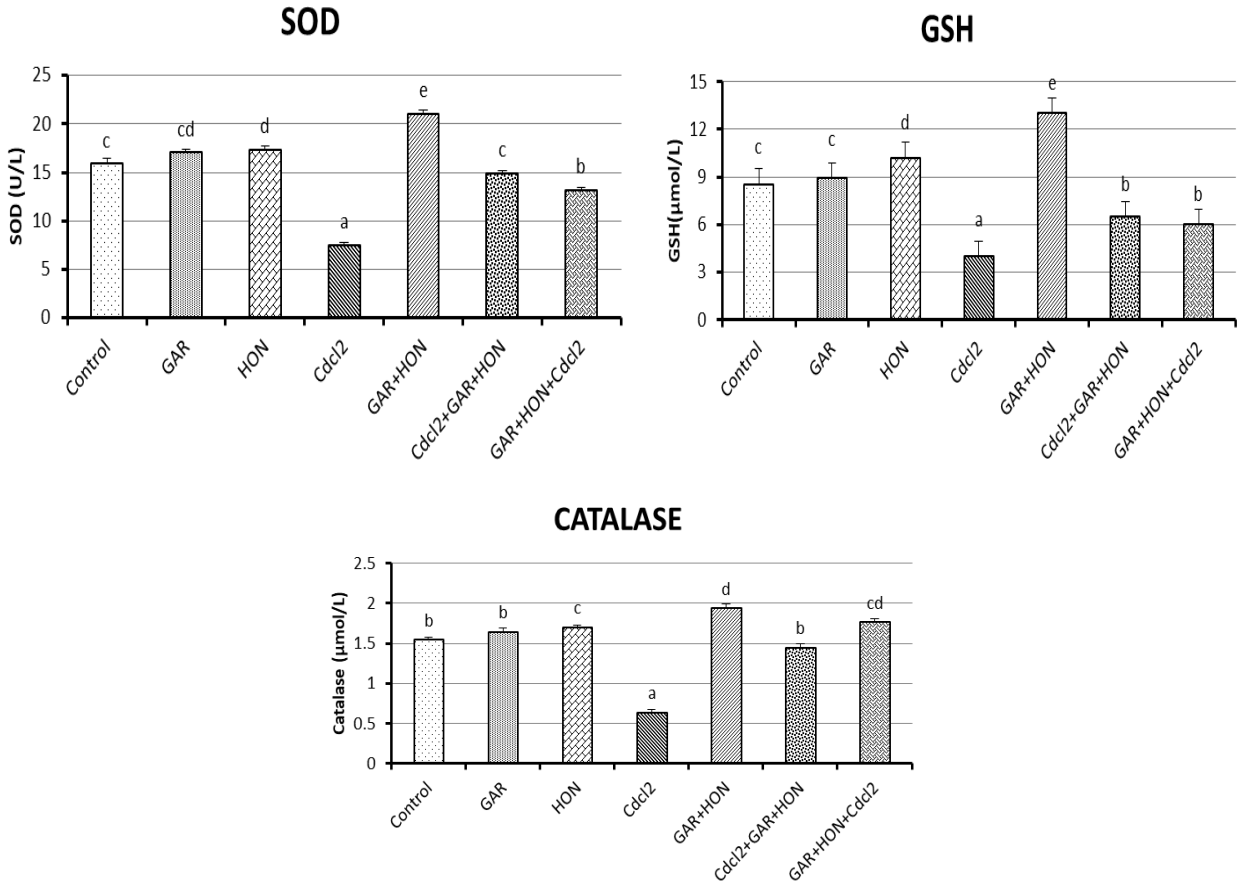

Fig. 3. Effects of cadmium and protective role of GAR + HON in SOD, GSH and CAT in blood serum of male rats.The results expressed as (Mean ± SE)
abcd = values at column with small different letters are different significantly at (P<0.05)

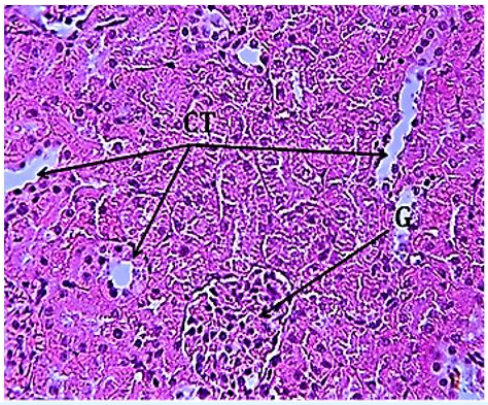

Fig. 4. Histopathologic findings in the Kidney of normal control rat (group 1) showing normal glomerular (G) and tubular histology (CT) (H & EX 400)

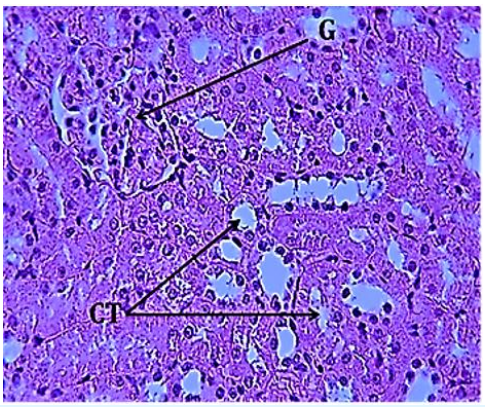

Fig. 5. Histopathologic findings in the Kidney of Garlic extract group showing normal glomerular (G) and tubular histology (CT) (H & EX 400)

($CdCl_2$ group) but GSH still lower than those of the control group while, SOD and CAT in group 6 were insignificantly changed, there was a significantly decreased in SOD and increased in CAT in 7th group when compared with control group as addressed in **Fig. 3**.

Histopathological results

The histopathological results of rats 4 groups of showed that kidney for first group showed normal complete architectures and details of tissue as illustrated in **Fig. 4**. Groups (2 and 3) treated only with garlic extract and honey, respectively exhibit renal tissues of no toxicity signs either tissue damages or hemorrhages as illustrated in **Figs. 5**

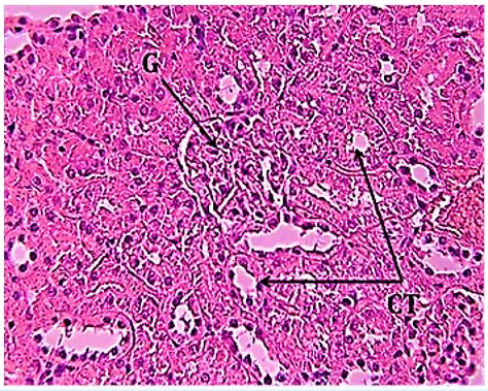

Fig. 6. Histopathologic findings in the Kidney of honey group showing normal glomerular (G) and tubular histology (CT) (H & EX 400)

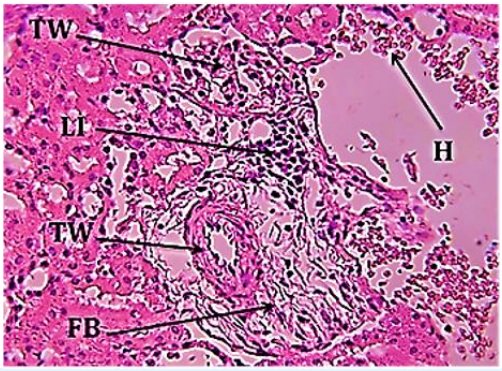

Fig. 7. Histopathologic findings in the Kidney of $CdCl_2$ group showing the glomerulus degeneration (DG), thickens of the vessel wall (TW), infiltration of inflammatory cells (L1), hemorrhage (H) and Presence of fibroplasts (FB) (H & EX 400)

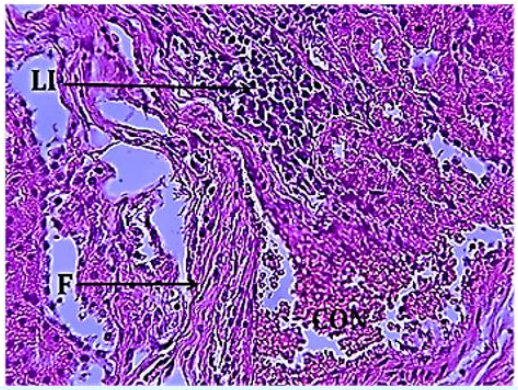

Fig. 8. Histopathologic findings in the Kidney of $CdCl_2$ group showing the bloody congestion (CON), infiltration of inflammatory cells (L1) and the presence of renal fibrosis (F)(H & EX 400)

and **6**. On the other hand, Group (4) treated with ($CdCl_2$) showed severe toxicity tissue exhibited obvious damage with congested glomeruli, severe inflammatory Lymphocytes infiltration, thickens of The vessel wall, hemorrhage, Presence of fibroblasts and degeneration of renal tubules and Bloody congestion **Fig. 7** and **8**, while Group (5) which treated with Garlic and Honey exhibited normal glomerular and tubular histology as illustrated in **Fig. 9**. Group (6) that treated firstly with Garlic + Honey and

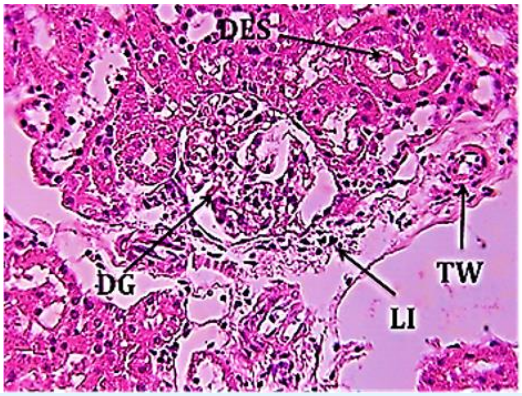

Fig. 9. Histopathologic findings in the Kidney of GAR+ Honey then $CdCl_2$ group showing the glomerulus degeneration (DG), thickens of the vessel wall (TW), infiltration of inflammatory cells (L1) and (DES) (H & EX 400)

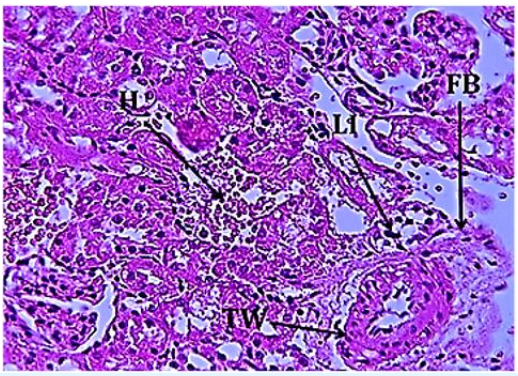

Fig. 10. Histopathologic findings in the Kidney of GAR+ Honey then $CdCl_2$ group showing thickens of the vessel wall (TW), infiltration of inflammatory cells (L1), hemorrhage (H), and presence of fibroblasts (FB) (H & EX 400)

then with $CdCl_2$ treated showed that The glomerulus degeneration, thickens of The vessel wall, Infiltration of inflammatory cells, (DES), hemorrhage and Presence of fibroblasts as in **Figs. 9** and **10**. Group (7) that treated firstly with Cd for Four weeks and then with Garlic + Honey extract treated revealed that tissue of renal is becoming completely being normal except mild degeneration presence in the proximal convoluted tubules lining epithelium as well as thickens of The vessel wall.

DISCUSSION

Changes being harmful in blood parameters were observed after 90 d administration of $CdCl_2$. Because kidney and liver are major organs involving in toxins elimination and transformation, their functions might disturb. Nevertheless, such changes mostly revealed improvement signs following GAR+HON treatments as aqueous extract.

Kidney urea, creatinine and UA levels are mostly detected as renal function indicators. Such measurements might furnish more information regarding renal function (Andjelkovic *et al*, 2019). Declining filtration of glomerular is proposed as result in the elevation levels of creatinine, serum urea, and UA attributed to elevation re-absorption in tubular (Yildirim *et al,* 2018) and this is in good agreement

with the present study. The results of the study conducted by (Vijaya & Sharma, 2018; Mohamed & Mohamed, 2009) to detect the protective potential of aqueous garlic extract on toxicity induced by Cd in mice. It was noted that the administration of aqueous garlic extract in conjunction with cadmium has a significant role in reducing the level of urea and creatinine in the blood of the group treated with cadmium. This is due to the aqueous extract of garlic containing phenols, polyphenols, flavonoids and antioxidants, these compounds can remove free radicals (OH •, 1O2, O2- •) from the body, especially glycoside, alkaloids, allicin, flavonoids and amino acid acids such as glutamine, cysteine and Glycine, as it can play the role of antioxidants, reduce oxidative damage to renal cells and glomeruli, increase glomerular filtration rate, and reduce the damage caused by free radicals (Nasiri *et al,* 2017). A study conducted by (Abdulaziz *et al,* 2013) that the levels of UA, urea, and creatinine were decreased significantly resulting from treating cadmium-exposed mice with honey compared to the control level. According to a study conducted by (Atagana & Asagba, 2014), honey effect on levels of uric acid in the rats plasma exposed to chronic and acute cadmium, honey administration with cadmium resulting in a significance decrease ($P<0.05$) in uric acid in the plasma of mice after acute and sub-chronic exposure to cadmium in comparison to groups. Increased formation of purines, which are essential substances for the formation of uric acid, which may result from the destruction of nucleic acids, as cadmium breaks down DNA (Atagana & Asagba, 2014).

In this study, facts in which were demonstrated via increased NO and fractional urea excretion levels. Whereas increased Garlic vasodilator effect was depicting via increased levels of kidney and plasma NO, the increase Garlic -induced in renal perfusion was corroborating through a significant elevation in FE urea followed by administration of Pb. Based on literature, NO considered as a potent vasodilator which was reported being crucial for normal function of kidney because of its important function in mechanisms of renal include release of renin, regulation of extracellular fluid, feedback of tubuloglomerular along with medullary and glomerular hemodynamics regulation. Garlic administration associated with renal clearance normalization through improving system of antioxidant and lowered significantly peroxidation of kidney lipid. It is obvious; such express the extract as a therapeutic potential choice in treatment of adjuvant or patients management with renal OS which is correlated with reperfusion injury of renal ischemia. The pharmacological extract activities as demonstrated via potentials antioxidant-boosting and vasodilatory were allow via its significance phytochemicals i.e., phenolics, flavonoids, tannins and alkaloids.

In an acute rats study conducted, one rats group was treated orally with a single 30 mg Cd/kg B.W. dose showed declined activity of SOD in groups treated with Cd compared to values of control which might due to noticed decline in concentrations of blood Zinc under conditions of experimental and observe increased in other parameters investigated, such as status of total oxidative, O^{2-}, levels of MDA and advanced oxidation content protein products, was more profound compared with control group (Matović *et al*, 2015). (Mohammed *et al,* 2014) attributed the high level of MDA to the generation of free radicals as a result of treatment with CdCl2 that oxidizes lipids in cell membranes. As unsaturated fatty acids of cell membranes are the target of the most vulnerable to free radical reactions because they have double bonds that are the main target of free radicals, MDA is produced by oxidation of these fatty acids through free radical reactions of the lipid peroxidation process. The results of (Ayakeme *et al,* 2012) agree with the current results, as it was demonstrated that garlic extract has an effect on cadmium-induced toxicity in mice. And that the treatment with it significantly reduces the levels of lipid peroxide (MDA). The reason for the decrease in MDA is due to the aqueous extract of garlic containing natural antioxidants represented by alkaloids as well as the presence of vitamins B1, B2, B6, C, and E) in addition to its containment. Important phenolic compounds (YAN & CHEN, 2017), which all work either to remove free radicals directly by giving them an electron or work to remove them indirectly by increasing the activity of antioxidants such as catalase (CAT), superoxide dismutase (SOD) and clutathione (GSH). Which all act to inhibit the oxidative potential and inhibit MDA production (Ayakeme *et al,* 2012). (Çavuşoğlu *et al,* 2009) Reported that the oxidative damage caused by Cd caused a significant increase in the level of MDA in the kidneys and that honey treatment contributed to significantly inhibiting MDA production. It can be concluded that honey has a protective role against Cd-induced oxidative stress in mice, because honey has antioxidant activity mediated by different mechanisms, as honey neutralizes free radicals after their formation. On the other hand, honey contains antioxidants represented by catalase and non-enzymatic compounds represented by flavonoids and polyphenols. Honey has different forms of flavonoids, which are pinobanksin, chrysin, and kaempferol. It also has effectiveness against oxidation as it protects cells from the influence of free radicals and maintains Cellular vitality, as honey is rich in vitamin C or so-called ascorbic acid, its percentage in honey is three times its percentage in human blood serum and has a role in the antioxidant defense system (Dżugan *et al,* 2018).

Analyses as histopathological showed marked glomerulus atrophy and degeneration followed via hyperemia, the formation of pyknotic cell in the tubules, The lesions being pathological created through Cd were reduced remarkably by Cd + Garlic extract being pre and post administration and revealed the renal tubules and glomeruli seemed to be similar as that of standard whereas renal medulla revealed amelioration being partial with little shrinkage in the formation of pyknotic nuclei and lumen. Honey exposes the activity of strong antioxidants (Renugadevi and Prabu, 2009). Such antioxidant honey capacity contributes to several chronic disorders and acute prevention i.e., allergic, inflammatory, thrombotic, cancer, cardiovascular, diabetic, and others (Abdel-Moneim and Ghafeer, 2007). Honey from different countries and floral origin exhibited high antioxidant characters. Flavonoids and phenolic acids are of responsibility for well-established honey antioxidant activity and inhibition of the lipoprotein

oxidation. Different polyphenols are mentioned in honey. Some honey polyphenols i.e., Chrysin, Caffeic acid, Galangin, Caffeic acid phenyl ester, Acacetin, Quercetin, Kaempferol, Pinobanksin, Apigenin, and Pinocembrin have evolved as agents promising being pharmacological in cancer treatment(Ali and Rajab).

Garlic and honey are powerful antioxidant properties, were mixed to give a synergistic or additive effect for their antioxidant efficacy.

CONCLUSION

Treatment of CdCl2-intoxicated animals with aqueous garlic extract and honey revealed a decline in the harmful Cd influences via restoring antioxidant activities, biochemical modifications, and oxidative stress markers.

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