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Effects of bee glue on oxidant-antioxidant balance and renal function in the male rat exposed to oxidative stress induced by hydrogen peroxide

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Abstract: This study was carried out from January to March 2024, aimed at assessing the impact of bee glue extracts (aquatic and ethanolic) on the oxidant-antioxidant balance by estimating the concentration of certain antioxidants and their indicators such as glutathione (GSH) and malondialdehyde (MDA), as well as assessing their impact on kidney functions by estimating the concentration of urea, creatinine, Blood urea nitrogen (BUN), BUN/Creatinine ratio and creatinine clearance (CrCl) among male rats exposed to hydrogen peroxide-induced oxidative stress. The results showed that treatment with bee glue extracts led to an increase in GSH, BUN/Creatinine ratio and CrCl levels in serum and a decrease in MDA, urea, creatinine and BUN levels compared to the H₂O₂ group. The study results indicate that both aqueous and alcoholic extracts of bee glue have a preventive role against oxidative damage caused by stress. This is achieved by improving the levels of GSH, BUN/Creatinine ratio and CrCl levels and reducing the level of lipid peroxidation (MDA). Additionally, the kidney function is enhanced by reducing the levels of urea, creatinine and BUN in rats treated with H₂O₂.

Keywords: *Glutathione, Malondialdehyde, Propolis, Blood Urea nitrogen, CrCl, BUN/Creatinine ratio*

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Introduction

Oxidation processes occur naturally in living cells with the participation of oxygen (O₂) in carbon-containing molecules for the purpose of generating energy to conduct vital activities, and at the same time they cause the formation of secondary oxidants known as free radicals (FRs) [1]. The accumulation of these compounds Oxidants, including free radicals, inside the body in high concentrations exceed the ability of antioxidants (AO) to remove them, and their interaction with various biological compounds leads to damage inside the body, and the occurrence of oxidative stress (OS) [2] who destroys cellular molecules in the body [3], making him a major cause in many diseases, such as diabetes, cancer, cardiovascular disease, arthritis, Parkinson's diseases, Alzheimer's diseases and kidney diseases [4].

Free radicals are usually disposed of and its effects by the use of antioxidants described as molecules that prevent or delay the oxidation of biological particles by working to remove free radicals or by modifying the cellular processes responsible for the production of free radicals [2]. Some of the antioxidants are manufactured and have harmful side effects, and others are naturally found in medicinal plants, vegetables, spices, fruits, and microorganisms [5] and the honey bee product known as bee glue [6], which is defined as a natural gummy product, complex in composition, consists of a mixture of beeswax, resinous substances, pollen grains and essential oils collected by honey bees from

tree buds and bark, and produced after treatment and mixing with enzymes [7] It includes among its components many secondary metabolites such as flavonoids, phenolic compounds, alkaloids and bioactive saponins, which stimulate its various effects [8] especially its action as a powerful antioxidant [9].

Aim of study:

Based on the above, this study came to achieve a set of aims, respectively:

1. Evaluating the effect of bee glue on the level of oxidative stress by measuring the level of antioxidants
2. Evaluation of the effect of bee glue on kidney function by estimating the concentration of creatinine , urea ,BUN,BUN/Creatinine ratio and CrCL and it is used to indicators of kidney function.
3. To evaluate the effect of the type of solvent used in the extraction process on the effectiveness of bee glue extracts on the variables studied.

1. Materials and Methods

2.1. Materials

Several tools and devices were used to conduct the experiment, which is:

2.2. Animals used in the study

A number of twenty-five male white the Sprague Dawley rats, weighing between 160-200 g, were collected from the Faculty of Veterinary Medicine at the University of Tikrit. The rats were housed in plastic cages measuring (46x28x13cm), under controlled

Tools and devices	Contry of origin	Tools and devices	Contry of origin
Electric blender	Germany (Silver crest)	Dissection kit	China (Huawei)
Rotary evaporator	England (Stuart)	Blood Glucose Monitor	Germany (Precicheck)
Electric oven	Germany (Mettler)	Basic laboratory tools	China
Spectrophotometer	USA (JASCO)	Hydrogen peroxide	Germany (Mychem Gmbh)
Incubtar	Germany (Mettler)	Ethanol alcohol	Spain (Scharlau)
Centrifuge	England (GallenKamp)	Chloroform	India (Himedia)
Water bath	Germany (Mettler)	Ready-made analysis (Kits)	Switzerland (Aggappe Diagnosis)
Vortex	England (Griffin)	Dissection kit	China (Huawei)
Whatman no.1	China (Jiao Jee)	Blood Glucose Monitor	Germany (Precicheck)

conditions of temperature (25°C) and lighting (12 hours of light and 12 hours of darkness). The rats were provided with good ventilation throughout the 21-day experimental period.

2.3. Experiment design

Four groups were assigned to the animals: Until the completion of the trial period, the first group (control) received nothing but food and water. The second group received a 21-day dose of H₂O₂ in drinking water at a concentration of 0.5%. the third group received a dose of 0.5% H₂O₂ and a 21-day course of treatment with 4% concentration of the aquatic extract of bee glue (A.E.P.), 4- The fourth group received the alcoholic extract treatment and an H₂O₂ dosage in 0.5% concentration drinking water.

2.4. Collection of samples bee glue and Preparing the extracts

The samples were collected directly from the honey beehives *Apis melifera* and kept after collection in plastic containers in the refrigerator (freezer) and under a temperature of (-20) °C to become solid and grindable using an electric grinder. After that, the method of [10] for the preparation of Ethanolic extract of propolis(bee glue) (E.E.P) and the method of [11]for the preparation of aqueous extract (A.E.P) were adopted.

2.5. Determination of active dose

A method [12] were used to determine the most effective dose for both the aqueous extract and the alcoholic extract of bee glue , the appropriate dose of of hydrogen peroxide to urge oxidative stress in the males of the rats was determined based on previous studies including the study of [13].

2.6. Collection of blood samples

The animals fasted for 12 hours, and then drugged using chloroform and blood samples were drawn through the heart directly and placed in test tubes and the tubes were transferred to the incubator for 30 minutes and under a temperature of (37C°) and separated the serum from the rest of the blood components using a centrifuge at a speed of 3000 cycles / minute for 15 minutes for the purpose of and used a micropipettes to withdraw the serum and keep it in deep freeze and at a temperature of (-20 C°) until the required tests are performed.

2.7. Estimation of the variables studied

The levels of enzymatically studied variables were estimated using the number of kits of the Switzerland company Aggape and according to the method of action attached to each kit as glotathione according to the method of [14]. Malonedialdehyde according to method of [15], the urea and BUN according to method the [16] and the creatinine according to method [17].

2.8. Statistical Analysis

The statistical analysis was conducted using the SPSS software. Morale was assessed using the Anova-One Way test, and significant differences were determined using the Duncans Multiple Ranges test ($p \leq 0.05$) [18].

2. Results

2.1. Glutathione and malondialdehyde

The results of the study shown in Table 1 indicated a significant decrease in glutathione concentration and a significant increase in malondialdehyde (MDA) concentration in the peroxide group (group 2) compared to the control group. In contrast, the other groups treated with propolis showed a significant increase in glutathione concentration and a decrease in MDA.

Table (1) The effect of the studied coefficients on oxidative stress induced by hydrogen peroxide in male rat

Groups Param	Control	H ₂ O ₂	H ₂ O ₂ +A.E.P	E.E.P H ₂ O ₂ +
GSH (μmol\L)	12.26 ±0.39 a	11.57±0.16 b	12.25±0.33 a	12.46±0.56 a
MDA (μmol\L)	29.84±0.08 c	59.75±1.49 a	32.92±3.05 bc	32.04±1.50 b

Note : Different letters horizontally indicate a significant difference at the ($p \leq 0.05$)

2.2. Kidney Funication (Urea , Creatinine, BUN, Creatinine clearance , BUN/Creatinine ratio)

The results of our study, shown in Table 2, indicate a significant increase in urea, creatinine, and Blood Urea Nitrogen (BUN) levels and a significant decrease in Creatinine clearance and the BUN/Creatinine ratio in the second group dosed with hydrogen peroxide compared to the control group. In contrast, the groups treated with propolis extracts showed opposite results when compared to the second group.

The effect of the studied coefficients on the kidney function Table (2)

Groups Parameter	control Group	H ₂ O ₂ Group	H ₂ O ₂ +A.Q.P Group	H ₂ O ₂ +E.E.P Group
Urea (mg/dL)	47.25±3.5 c	55.6±4.33 a	48.18±1.7 bc	44.04 ±1.98 c
Creatinine (mg/dL)	0.30±0.02 c	0.36±0.03 a	0.29±0.01 c	0.15±0.02 d
Blood urea nitrogen (BUN) (mg/dL)	23.02±1.63 c	26.89±2.01 a	23.45±0.77 bc	21.51±0.94 c
BUN/Creatinine ratio	87.38 ± 7.22 b	77.41±2.31 c	84.56±0.82 b	153.73±2.2 a
Creatinine Clearance (ml/min)	0.87±0.05 b	0.78±0.02 c	0.89±0.03 b	1.44 ±0.08 a

Note : Different letters horizontally indicate a significant difference at the ($p \leq 0.05$)

3. Discussion

3.1. Glutathione (GSH) and Malondialdehyde (MDA)

The non-protein thiol glutathione is found in mammalian tissues in millimolar quantities. It is a significant intracellular antioxidant that regulates the redox state of cells and shields them against xenobiotics, reactive oxygen and nitrogen species, and lipid peroxides [19] as for malonaldehyde (MDA) it can be described as the final product of lipid peroxidation by free radicals [20] main bio results of our current study showed a significant decrease ($P \leq 0.05$) in the levels of GSH and a moral rise in the MDA concentration in the group of hydrogen peroxide when compared to the control group. This results came in compliance with the results of [21] that which It indicated that there is a moral decrease in some antioxidants such as GSH and a moral rise in the MDA concentration as a result of an increase in oxidative stress, which causes increased free radical formation and antioxidant drain.

The decrease in GSH concentration in the hydrogen peroxide-treated group is due to its involvement in oxidation-reduction reactions. Hydrogen peroxide increases the production of free radicals, resulting in the depletion of antioxidant enzymes, especially reduced glutathione from blood and tissue [22]. Reduced glutathione is primarily formed due to the activity of the enzyme glutathione reductase, with NADPH from the pentose phosphate pathway serving as a cofactor, which is negatively affected by increased production of free radicals [23], While the reason for the significant rise of MDA is due to the imbalance of the balance of oxidation - antioxidants in pathological cases in favor of free radicals, which encourages them to attack and oxidize fatty acids PUFAs present in the phospholipid layers of cell membranes [24] producing Lipid hydroperoxides, the most important of which is MDA, which is characterized by its high toxicity to cells and its ability to inhibit antioxidant enzymes [25]

The results of the study show a significant increase in GSH in the groups treatment with the bee glue extracts, when compared to the group of hydrogen peroxide. This result came compatible with the results of both [26] and [27] In addition to the study of [28], which referred to the role of alcoholic extracts of bee glue in the events of a moral increase

in the levels of antioxidants such as GSH. The significant increase that occurred after dosing with alcoholic and aqueous extract is due to the chemical composition of bee glue, which is characterized by containing many bioactive chemical compounds (more than 850 compounds [29] with various biological and therapeutic properties, especially their ability as antioxidants, the most important of which are flavonoids, phenolic compounds, phenethyl, caffeic acid CAPE and coumaric acid... [30] Which indirectly contribute to the reduction of oxidative stress by inhibiting enzymes that participate in the formation of ROS reactive species such as mono-oxygenase and NADPH-oxidase enzymes or directly by capturing reactive species to maintain antioxidant concentrations, and this is what flavonoids [31] do in particular because of their ability to donate electrons from the hydroxyl OH group present in its composition to unstable ROS reactive species [32]. Phenethyl caffeic acid (CAPE) is also one of the compounds available mainly in bee glue, as well as its presence in natural materials such as plants and fruits [33] and has a protective role in cells from oxidative stress damage [34], Sezgi *et al* indicated its role in protecting the lung tissue from the oxidative damage caused by radiation by reducing oxidation factors and increasing the production of antioxidants, by increasing the expression of anti-oxidant enzymes and inhibiting the expression of inflammatory cytokines and adjusting the nuclear factor paths [35] with a pivotal role in organizing genetic expression of antioxidants [36] Especially expressing Heme Oxygenase 1 (HO1) that activates cellular defense mechanisms against oxidative stress [37].

The decrease in the concentration of MDA in groups treated with bee glue extracts is mainly due to the increased production of antioxidant enzymes and the inhibition of the action of enzymes forming reactive oxygen species due to some biologically active compounds in bee glue [31], which leads to reducing lipid peroxidation and ultimately reducing the production of malonyl dihydrate. Aldehyde. Many studies have indicated the role of bee glue supplements in influencing fat metabolism and oxidative stress by reducing the products of the fat oxidation process such as thiobarbituric acid reactive substances and increasing antioxidant levels [38]. The results also showed that the effect of bee glue on antioxidants was higher in the alcoholic extract compared to the water extract, because its phenolic content extracted with alcohol is ten times higher compared to the extract with water [39]. This is due to the fact that the majority of its active compounds are poorly soluble in water [40],

3.2. Urea and Creatinine

Table 2 shows a significant increase ($p \leq 0.05$) in the concentrations of urea, creatinine and BUN in the hydrogen peroxide-treated group compared to the control group, due to damage to renal tissue, or internal functional damage that impairs kidney function and affects its functioning and Creatinine can be defined as a vital biomarker used to assess kidney function and diagnose acute kidney injury (AKI), It is a waste product that is produced by the body and excreted by the kidneys. Elevated serum creatinine levels can indicate impaired kidney function, which is a common complication in various medical conditions, such as cardiac surgery and dialysis [41] as for urea, it is produced in the liver through the breakdown of amino acids, primarily from the metabolism of dietary proteins. The liver converts these amino acids into urea, which is then transported to the kidneys through the bloodstream (43) (44).

Giving rats hydrogen peroxide with drinking water leads to increased oxidative stress and causing changes in kidney function, as exposure to hydrogen peroxide in some studies led to kidney injury, which results in increased creatine and urea concentrations in the blood [43] The oxidative stress and the accumulation of free radicals is one of the main causes of the high concentration of urea in the blood through its work on the production of urea as a secondary product to oxidize proteins and amino acids [44] As the high levels of oxidant reduce insulin secretion and thus impose on cells the oxidation of proteins and fats as an energy source, which increases urea levels in the blood serum (Table 2), as well

as increases the lipidperoxidation and pyroxy nitrite as a Giving rats hydrogen peroxide with drinking water leads to increased oxidative stress and causing changes in kidney function, as exposure to hydrogen peroxide in some studies led to kidney injury, which results in increased creatine and urea concentrations in the blood (51)[43] The oxidative stress and the accumulation of free radicals is one of the main causes of the highconcentration of urea in the blood through its work on the production of urea as a secondary product to oxidize proteins and amino acids [44] As the high levels of oxidant reduce insulin secretion and thus impose on cells the oxidation of proteins and fats as an energy source, which increases urea levels in the blood serum (Table 2), as well as increases the lipidperoxidation and pyroxy nitrite as a result of the increase in free radicals, in particular the radical of super oxide anion [45] increased creatinine concentration may also be attributed to damage to renal glomeruli, this allows creatine to be released into the blood [42].

3.2.2. Blood Urea nitrogen (BUN)

BUN is a relatively common routine test that can predict the risk of diseases caused by oxidative stress and contribute to the careful monitoring of any adverse vascular and renal events [46], Where the results of the study indicated a significant increase in the level of BUN in the H₂O₂ group; This may be attributed to renal dysfunction as a result of oxidative stress causing severe and dynamic impairment, metabolic disorder in kidney function, and damage to its tissues as a result of increased free radicals [47] Which may lead to several pathological and functional renal disorders, the most important of which is diabetic nephropathy [48].

The dosing of rats with bee glue caused a significant decrease in urea and creatinine levels, and this is consistent with the results of the study of [49] and the study of [50], which showed the positive role of bee glue extracts on kidney function and tissue. This is due to several reasons, including its antioxidant efficacy and its ability to neutralize the harmful effects of free radicals by reactivating natural defense mechanisms, which protects renal tissue from the negative effects of oxidative stress [51] bee glue was able to improve kidney structure and reduce glomerular swelling compared to untreated renal tissue through its anti-inflammatory action by inhibiting and reducing the regulation of a number of pro-inflammatory agents and cytokines such as interleukin IL-1 β , IL-6, IFN- γ and TNF- α or through its action in reducing the migration of immune cells such as phagocytes and neutrophils [52] in addition to his its in reducing the programmed death of renal cells exposed to oxidative stress by regulating the expression of Caspase-9 [53]

Bee glue treatment leads to a significant improvement in kidney function and leads to a strong effect in getting rid of free radicals because of its antioxidant compounds [54], and significantly reduces the percentage of BUN[55] This is consistent with the results of our study , It points to the effective role of aqueous and alcoholic bee glue extracts in improving kidney function by reducing levels of urea, creatinine and blood urea nitrogen in serum for male rats exposed to oxidative stress.

3.2.3. BUN/Creatinine ratio and creatinine clearance (CrCl)

BUN/Creatinine ratio is an important and effective biomarker widely used in clinical cases to assess kidney function and disease severity in different cases and this ratio is easily calculated by dividing the level of urea nitrogen in the blood by the level of creatinine (56).

The results of our study shown in Table 2 showed a significant decrease in BUN/Creatinine ratio and in the creatinine CrCl clearance rate of the hydrogen peroxide group (group II) compared to the control group.

The decrease in BUN/Creatinine ratio in the peroxide group may be due to metabolic processes and creatinine level regulation being affected by oxidative stress induced by peroxide, and the latter may lead to direct kidney damage and lower glomerular filtration

rate [41] which may increase creatinine levels more than BUN. As a result, the rate of urea nitrogen on creatinine decreases. As for the totals treated with bee glue, a significant increase in the values of this variable may be attributed to several reasons, including the treatment and preventive effects of bee glue of oxidative damage on the liver and its ability to improve liver function, including his participation in the production of urea [56] Which affects the value of Bun/creatinine ratio positively, and this result may be attributed to the role of bee glue in improving the efficiency and function of the kidneys in general and the glomerular filtration rate in particular for both creatinine and urea.[50] [49].

Creatinine Clearance (CrCl) is defined as the volume of blood plasma that is completely purified of creatinine per unit of time (e.g. ml/min) and is usually estimated using serum creatinine level along with factors such as age, sex and body weight [57]. The results in the second group showed a decrease in the CrCl in the serum. This may be due to damage to the renal glomeruli as a result of exposure to oxidative damage caused by oxidizing agents, including hydrogen peroxide [42], while the groups treated with bee glue showed a significant increase in the creatinine clearance rate., when compared to the second group, is due to its ability as an antioxidant and its effectiveness in neutralizing the harmful effects of free radicals by reactivating natural defense mechanisms and thus protecting the renal tissue from the negative effects of free radicals [51], as well as its ability to improve the kidney structure. It reduces swelling of the glomeruli through its anti-inflammatory action by reducing the production of a number of pro-inflammatory cytokines and reducing the migration of immune cells [52].

4. Conclusion

We concluded from the results of this study that bee glue has an effective and positive role in reducing the accumulation of free radicals directly through the ability of its active compounds to reduce or indirectly through activating and increasing the expression of antioxidants such as glutathione, which leads to reducing oxidative stress and the resulting diseases that may affect the body's organs such as the kidneys, and this was evident through its ability to increase GSH, BUN/creatinine ratio and CrCl levels and reduce MDA, urea, creatine and BUN levels. In addition to the above, the results of this study confirmed that the effectiveness of bee glue has varied according to the type of solvent used in the extraction process, where the alcoholic extract (ethanol) showed the strongest positive effect on the studied transactions compared to the aqueous extract and this may be due to the fact that bee glue consists of a large proportion of waxy materials, volatile oils and other materials with little solubility in water and non-polar. It is chemically known that polar substances dissolve in polar solvents such as water and non-polar substances dissolve in non-polar solvents such as ethanol and acetone.

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