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Study effect of crude alcoholic extract of Hawthorn (Crataegus *Oxyacantha*) and crude polyphenolic compounds from blackolive (*Olea Europae*) fruits on some physiological parameters of kidney of male rats treated with hydrogen peroxide

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Abstract:

This study was carried out to investigate the protective role of 70% ethanolic alcohol extract of Hawthorn (crataegus oxyacantha) and crude polyphenolic compounds of black olive fruits (oleae uropae) on some physiological functions of the male rats kidney treated with 1% hydrogen peroxide .Twenty mature male newzeland rats were divided randomly into four equal groups and treated for 30 days as follows:-control group which were given ordinary tap water , first treated group (G1) was given 1% H2O2 in drinking water , the second treated group (G2) was orally given alcoholic extract of Hawthorn 300 mg / kg B.W. with 1%hydrogen peroxide H2O2in drinking water , third treated group(G3) was given 200mg /kg B.W. of crude extract of black olive with 1% H2O2 . Blood samples were collected at zero time and 30 days of the experiment for measuring the following parameters: - concentration of uric acid,urea,creatinine and glucose. The results showed significant increased in the concentration of uric acid and glucose of the group treated with 1%hydrogen peroxide H2O2 (G1) and significant decrease in the concentration of uric acid, creatinine, glucose of treated groups G2and G3, also significant decrease in the concentration of urea of treated group (G3) as compared with control group. Histological study revealed that oral treatment with 1%H2O2 caused cell necrosis and vacuolar degeneration of renal epithelial cells. It is concluded that treatment with hawthorn and crude polyphenol showed no clear pathological lesions. The present study documented the deleterious effect of H2O2 and renoprotective effect of Hawthorn and black olive fruits.

Key words: *-Hawthorn, olive oil, ROS, H2O2, free radicals, oxidative stress.*

Introduction:

Hydrogen peroxide is a common oxygen radical capable of causing significant cellular damage even at low concentrations (1, 2). The oxygen radical can ultimately cause oxidative stress, resulting in significant cellular and intracellular damage and necrosis (3, 4, and 5). Oxidative stress is a state of imbalance between free radicals production and its degradation by antioxidant systems with increased accumulation of the radicals over 90% of reactive oxygen species (ROS) formation occurs in mitochondria during metabolism of oxygen when some electrons passing the electron transport chain may leak from the main path and can directly reduce oxygen molecules to the superoxide anion (6,7) .In the body superoxide dismutase catalyses the formation of oxygen and hydrogen peroxide; the enzyme catalase is then responsible for reacting with the hydrogen peroxide (H2O2) species, to ultimately form water and oxygen. (3, 4, 5, 8, 9). Oxidative stress has been showed important pathologic mediators in kidney disease .Studies in patient with varying degree of kidney impairment suggest that patients with chronic renal disease are in state of oxidative stress compared with healthy controls, and the degree of oxidative stress is correlated with degree of renal failure (10, 11). Antioxidant therapy might be an important tool in the treatment of free radicals-mediated disorders (12, 13).Hawthorn (crataegus oxycantha) and olive fruit(oleae uropae) among a variety of herbs and medical plants that show significant antioxidant properties (14 , 15 , 16 , 17 , 18). This study was designed to investigate the

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protective role of Hawthorn and black olive fruits on kidney function tests in males rats exposed to hydrogen peroxide over load.

Materials and Methods:

The fresh fruits of hawthorn were extracted with 70 % ethanol according to (19). , and the extraction of polyphenolic compounds from olive fruits was carried out according to Markham method (20) by using 95% methanolic alcohol (1: 9) and shaking the mixture, by using magnetic stirrer for 18 hrs. at room temperature, then filtrate with filter paper and concentrated the supernatant at 40°C in an incubator. the yield was brown pasty substance(creamy texture) that kept at –20°C till use .Twenty mature (3-5 months) adult Albino Wister male Rats were randomly divided into four groups (each of 5) and treated as follows for 30 days :- Animals in group one had free access to food and water and served as control , group two (G1) animals were subjected to ad libitum supply drinking water containing 1% H2O2 (35% of hydrogen peroxide solution was diluted with water , group three (G2) rats were subjected to ad libitum supply drinking water containing1% H2O2 and received 300mg/ kg B.W. of crude ethanolic extract of crataegus oxyacanthga, group four (G3) rats were subjected to ad libitum supply drinking water containing1% H2O2 and received 200mg /kg B.W. of crude polyphenolic extract of olea europae dissolved in distilled water. Blood samples were collected by heart puncture technique at 0 time and 30 days of the experiment, serum collection by centrifugation (3000rpm) for 15minutes and frozen at – 20 C° until analysis. Serum samples were used for measuring the following parameters: - serum uric acid concentration was enzymaticlly measured using enzymatic assay kit (linear chemicals) (21). Enzymatic and colorimetric methods were used for determination serum creatinine, serum blood urea nitrogen and serum blood glucose concentrations (22).The animals were then sacrificed for histological examination, and kidney tissue sections were prepared according to (23). Differences between experimental groups were statistically evaluated using two way analysis of variance (ANOVA) as described by (24).

Results:

The effect of70% alcoholic extract of crataegus oxyacantha and95% ,methanolic alcohol of polyphenolic compound from olive fruit and 1%H2O2on kidney function tests (uric acid , urea , creatinin)and on blood glucose concentration in mature male rats was shown in tables (1 ,2, 3 , 4) . Data pertaining to uric acid concentration showed in table (1). The results showed after 30 days of treatment, significant ($P<0.05$) increase in serum uric acid concentration in group (G1) as compared to control group and significant ($P<0.05$) decrease in serum uric acid concentration in group (G2), (G3) as compared to the treated group (G1). table (2) showed significant ($P<0.05$) decrease in blood urea concentration in treated group (G3) after 30 days treatment as compared to control and two others treated groups. Besides there were no significant differences ($P>0.05$) in this parameter in group (G1) and (G2) as compared to control one. The mean values of BUN in control, G1, G2groups at end of experiment were (39.74± 1.69), (41.43± 1.39), (39.67±1.99) respectively. Within the time, significant decrease ($P<0.05$) in BUN concentration in (G3) at day 30 was observed comparing to the data at day zero. Table (3) showed significant ($P<0.05$) decrease in serum creatinine concentration in treated groups (G2) and (G3) as compared to G1 and control groups after 30 days of treatment, also the results showed no significant differences($P>0.05$) between treated group G1and control group at the same period . The mean value of serum creatinine concentration showed a significant ($P<0.05$) decrease within the time in treated group (G2) and (G3) comparing to pretreatment period. There were no significant differences ($P>0.05$) in serum glucose concentration among experimental groups in pretreatment period (Table 4). After 30 days of treatment a significant ($P<0.05$) increase in serum glucose concentration was detected in 1% H2O2 treated group G1 as compared to control group , while the value in groups(G2) ,(G3) showed a significant ($P<0.05$) decrease in serum glucose concentration comparing to control group .

Histological changes: Exposure to 1% H2O2(group G1) showed inflammatory cells particularly neutrophils and macrophages infiltration around congested blood vessels, cell necrosis and vacuolar degeneration in epithelium cells of the renal tubules figure(2,3) comparing to the histological structure of normal kidney of control group figure (1). the histological changes in the kidney of rats of group (G2) treated with1% hydrogen peroxide H2O2 plus alcoholic extract of Hawthorn 300 mg / kg B.W and third group(G3) treated with1% H2O2 plus 200mg /kg B.W. of crude poly phenol of black olive fruit showed no clear pathological lesions figures (4, 5) .

Table 1: Effect of oral intubation of crude alcoholic extract *Crataegus Oxyacantha* (300mg/ kg B.W.),*Olea Europae* at(200mg /kg B.W.) and 1% H_2O_2 in drinking water on serum Uric Acid concentration (mg /dl)in male rats .

Groups / Days of treatment	Control group C	1% H_2O_2 G_1	crude extract of *Crataegus Oxyacantha* G_2	crude extract of *Olea Europae* G_3
Zero time	33.98± 2.33 Bb	41.63± 2.59 A	34.48± 2.16 B	39.24± 2.20 ABa
30 days	39.74± 1.69 Aa	41.43± 1.39 A	39.67±1.99 A	30.24±0.62 Bb

Values expressed as means ±SE.n=5/ group.
Capital letters denote between groups differences, P<0.05vs control.
Small letters denote within groups differences, P<0.05vs control.

Table 2: Effect of oral intubation of crude alcoholic extract Crataegus Oxyacantha (300mg/ kg B.W.), Olea Europae at (200mg /kg B.W.) and 1% H2O2 in drinking water on serum urea concentration (mg /dl) in male rats.

Groups / Days of treatment	Control group C	1% H_2O_2 G_1	crude extract of *Crataegus Oxyacantha* G_2	crude extract of *Olea Europae* G_3
Zero time	33.98± 2.33 Bb	41.63± 2.59 A	34.48± 2.16 B	39.24± 2.20 ABa
30 days	39.74± 1.69 Aa	41.43± 1.39 A	39.67±1.99 A	30.24±0.62 Bb

Values expressed as means ±SE.n=5/ group.
Capital letters denote between groups differences, P<0.05vs control.
Small letters denote within groups differences,P<0.05vs control.

Table 3: Effect of oral intubation of crude alcoholic extract *Crataegus Oxyacantha* (300mg/ kg B.W.), *Olea Europae* at (200mg /kg B.W.) and 1% H_2O_2in drinking water on serum creatinine concentration (mg /dl)in male rats

Groups / Days of treatment	Control group C	1% H_2O_2 G_1	crude extract of *Crataegus Oxyacantha* G_2	crude extract of*Olea Europae* G_3
Zero time	0.517± 0.059 B	0.717± 0.02 A	0.639±0.045 AB	0.609 ± 0.076 AB
30 days	0.574± 0.07 A	0.671± 0.08 A	0.405 ±0.02 B	0.449±0.05 B

Values expressed as means ±SE.n=5/ group.
Capital letters denote between groups differences, P<0.05vs control .
Small letters denote within groups differences ,P<0.05vs control.

Table 4: Effect of oral intubation of crude alcoholic extract *Crataegus Oxyacantha* (300mg/ kg B.W.), *Olea Europae* at (200mg /kg B.W.) and 1% H_2O_2in drinking water on serum glucose concentration (mg /dl)in male rats

Groups / Days of treatment	Control group C	1% H_2O_2 G_1	crude extract of *Crataegus Oxyacantha* G_2	crude extract of *Olea Europae* G_3
Zero time	209±14.6	238.8±17.4	202.2±12.19 A	219.2±20.11 a
30 days	182.59±8.03 B	276.48±21.91 A	136.71±4.47 Cb	172.15±5.52 BC b

Values expressed as means ±SE.n=5/ group.
Capital letters denote between groups differences, P<0.05vs control.
Small letters denote within groups differences, P<0.05vs control.

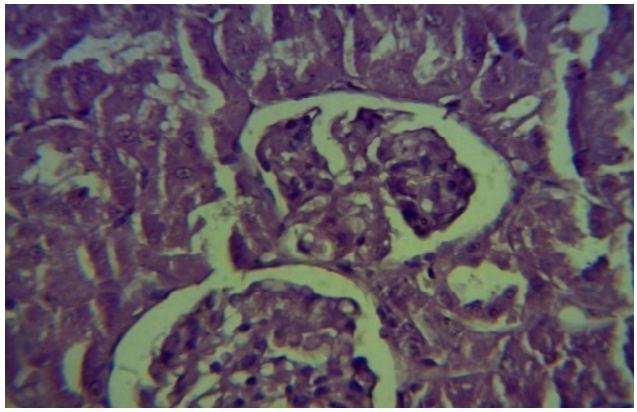
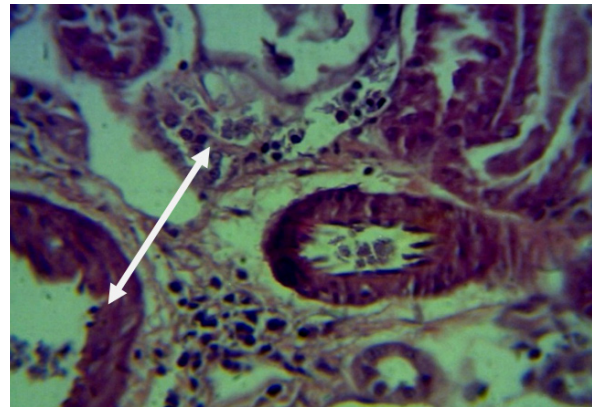


Figure (1): Histological section of normal kidney of rat (H&E, 40X)



Figure(2):- Histopathological section in the kidney of rat at day30 post treated with H2O2 shows inflammatory cells particularly neutrophils and macrophages infiltration around congested ⟷ blood vessels (H&E, 40X)

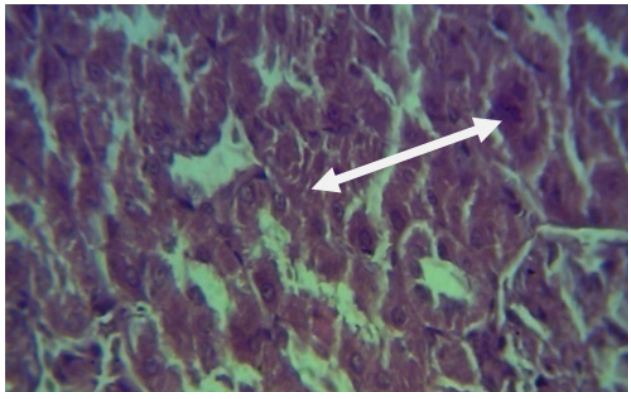


Figure (3): Histopathological section in the kidney of rat at day 30 post treated with H2O2 shows single cell necrosis ⟷ and vacuolar degeneration of renal epithelial cells (H&E stain 40x)

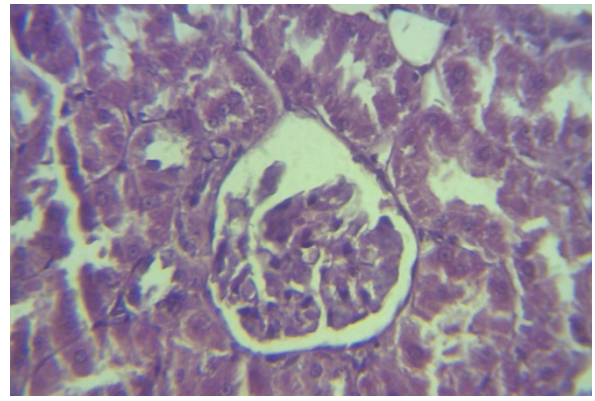


Figure 4: Histopathological section in the kidney of rat at day 30 post treated with H2O2 and hawthorn shows no clear lesions (H&E stain 40x)

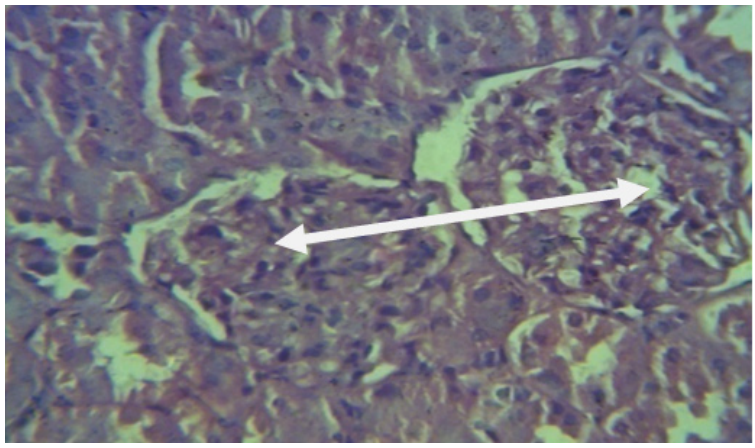


Figure (5): Histopathological section in the kidney of rat at day 30 post treated with H2O2 and polyphenol shows hypercellularity of glomerula ⟷

(H&E stain 40x)

Discussion:

According to the present study exposure to hydrogen peroxide (H2O2) in drinking water was found to cause a case of oxidative stress by many investigators (14, 17, 25), which may occur due to excessive generation of reactive oxygen species (ROS) or due to decreased ability of cells to scavenge the ROS as a result of defect in endogenous antioxidant defense system, leading to antioxidant depletion (26). The oxidative effect of H2O2 has been documented in various diseased conditions by many authors (27, 28). During heavy exposure to ROS, including H2O2 , the level of superoxide anion and other oxidants like H2O2 will increase tenfold with subsequent increased demand upon the antioxidant defense system of the body (29) . Recent evidence indicated that mechanisms of oxidative stress mediated by H2O2 injury may involve in the induction of gene expression which is regulated by nuclear transcription factor {(NF-kB), an oxidative stress responsive transcription factors} (30). The result of the present study revealed occurrence of renal disorder marked by elevation in uric acid in adult rat after exposure to 1% H2O2 in drinking water.

Oxidative stress (OS) markers and elements were studied in male rats to evaluate biochemically the degree of kidney damage, investigate the role of OS in the mechanism of functional renal disorders (31). Reactive oxygen species (including H2O2) are able to attack protein and lipids leading to membrane lipid peroxidation, and cellular dysfunction (32). We can hypothesized that exposure to H2O2 may cause elevation of superoxide anion and the dangerous hydroxyl (OH•) radical leading to glomerular dysfunction (33) Besides, NF-KB (induced in the study by H2O2 exposure) may lead to activation of a wide variety of inflammatory response like cytokines (34), thus diverse deleterious renal damage may occur with subsequent decrease in glomerular function which may result in elevation of kidney biomarkers.

The present study revealed significant elevation in serum blood glucose in experimental group administration 1% H2O2 .One potential central mechanism for glucose toxicity is the formation of excess ROS levels, which takes place within multiple mitochondrial and non-mitochondrial pathways. The islet is especially vulnerable to ROS because of its low intrinsic level of antioxidant enzymes. Chronically excessive glucose and ROS levels can cause decreased insulin gene expression via loss of the transcription factors PDX-1 and MafA and can also accelerate rates of apoptosis (35).Hyperglycemia increases the cell's susceptibility to oxidative stress and it also amplifies oxidative DNA damage (36).Oxidative stress plays a key role in the pathogenesis of insulin resistance and β-cell dysfunction (37). Overproduction of superoxide and H2O2, which, in turn, determine a decline in the antioxidant systems, directly damage many biomolecules; increase lipid peroxidation and results in insulin resistance (38).

Oral intubation of alcoholic extract of hawthorn and black olive fruit exert protective actions against damaging effect of H2O2 on renal system causing significant decrease in kidney biomarkers (uric acid, urea, creatinine) and significant decrease in blood glucose.

A number of studies have postulated that herbal extracts and plant derived active ingredients can protect body against oxidative stress (39). Some medicinal ingredients in plants may also act as antioxidants and protect the cell against the damage caused by ROS (40, 41).

Hawthorn contains a high proportion of polyphenolic compounds and exhibited good antioxidant activities. (42). many studies have shown that treatment of Crataegus. Oxyacantha significantly diminishes plasma glucose levels (43, 44), at the same time black olive fruit contain large amount of phenolic compounds like tyrosol, hydroxytyrosol, dihydrocaffeic acid, dihydro-p-coumaric acid (phloretic acid), acetoside (a disaccharide linked to hydroxytyrosoland caffeic acid), acetoside isomer and the flavonoids apigenin and luteolin.(45), these natural antioxidant may contributed to prevention of

oxidative stress effect (46 ,47). Previously, olea uropea was reported to have an anti-hyperglycemic effect (48) it inhibits hyperglycemia and oxidative stress induced by diabetes, which suggests that administration of olea uropea is helpful in the prevention of diabetic complications associated with oxidative stress (49).

Histological study showed that exposure to1%H2O2in drinking water showed cell necrosis and vacuolar degeneration of renal epithelial cell documented the renal damage effect of H2O2 (50),while treated with hawthorn and black olive fruits showed no clear lesions which is coincided with (51).It can be concluded that the hawthorn and black olive fruits have renoprotective effect.

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دراسة تأثيرالمستخلص الخام الكحولي لثمار الزعروروالمتعددة الفينول من ثمار الزيتون الأسودعلى بعض المعايير الفسلجية في كلى ذكور الجرذان والمعاملة بالبيروكسيد الهيدروجين

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الخلاصه:

أجريت هذه الدراسة لمقارنة الدور الوقائي للمستخلص الخام لثمار الزعروروالمستخلص الخام للمركبات المتعددة الفينول للزيتون الأسود في بعض المؤشرات الفسلجية في كلى ذكور الجرذان المعاملة ببيروكسيد الهيدروجين(1%) , أستعملت عشرون جرذا نيوزيلانديا من الذكور البالغة قسمت عشوائيا الى أربعة مجاميع متساوية وعوملت لمدة 30 يوم كالأتي :- مجموعة السيطرة (C)أعطيت ماء الشرب الأعتيادي , مجموعة معاملة ببيروكسيد الهيدروجين 1% (G1) وقد اعطيت ماء الشرب العادي المضاف اليه بيروكسيد الهيدروجين(H2O2) , مجموعة معاملة بالمستخلص الكحولي لثمار الزعرور مع بيروكسيد الهيدروجين(H2O2) ورمز اليها (G2) ومجموعة معاملة بالمستخلص الكحولي لثمار الزيتون الأسود مع بيروكسيد الهيدروجين (H2O2)ورمز اليها G3)) . تم سحب عينات الدم للفترات 0 و 30 يوم من التجربة لغرض ية اجراء الفحوصات التالية :-قياس تركيز كل من حامض اليوريك واليوريا والكرياتنين والسكر . أظهرت الدراسة الحالية أختلافا معنويا في مستوى تركيز حامض اليوريك والسكر في المجموعة (G1) وانخفاضا معنويا في مستوى تركيز كل من حامض اليوريك والكرياتنين والسكر في المجموعتين المعاملتين (G2) وG3)) كما وأظهرت النتائج أنخفاضا معنويا في مستوى تركيز اليوريا في المجموعة G3)) مقارنة مع مجموعة السيطرة . بينت نتائج الفحص النسيجي بوجود خلايا التهابية وحدوث تموت وتفجي هيولي في الخلايا المبطنة للنبيب الكلوي في المجموعة المعاملة (G1), في حين أدى تجريع الحيوانات بالمستخلص الكحولي للزعرور والمستخلص الكحولي المتعدد الفينولي لثمار الزيتون الأسود الى انكفاء التغيرات المرضية التي سببها بيروكسيد الهيدروجين .