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Protective role of alcoholic extract of black currant (*Vitis Vinifera* L.) on renal function of adult male rats exposed to methionine overload and hydrogen peroxide

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Abstract

This study was designed to investigate the effect of methionine overload in male rats on induction of renal damage comparing to an oxidant (Hydrogen peroxide).It also aimed to study the protective role of alcoholic extract of black currant on some biological markers related with kidney function in methionine overload and 0.5% H_2O_2 treated male rats. Fifty adult male rats were randomly divided into five equal groups (ten rats/ group) and were treated daily as follows for 42 days. Rats in the first group (C) were received 0.5 ml of buffer (0.1M, PH 7) by oral intubations and served as control group. Animals of the second group (T1) were received 0.5% H_2O_2 in drinking water, while rats of the third group (T2) were intubated orally 100 mg / kg B.W. of D.L. methionine diluting in buffer. Animals of the fourth group (T3) were received 0.5% H_2O_2 in drinking water plus (60 mg / kg B.W) alcoholic extract of black currant, while animals of the fifth group (T4) were intubated with same previous concentration of methionine and alcoholic extract of black currant. Fasting blood samples were collected from all experimental groups at 0, 21, and 42 days of experiment to study the following parameters: A- Serum glutathione (GSH) concentration (only at day 0 and 42). B- Serum creatinine (SC) concentration. C- Blood urea nitrogen (BUN) concentration. D- Serum uric acid (SUA) concentration and E-BUN / SC ratio. Sections of kidney were assessed for histopathological studies. The result revealed that exposure of animals H_2O_2 in drinking water(T1) or methionine (T2) for six weeks caused significant decrease ($P<0.05$) in GSH concentration and significant increase ($P<0.05$) in SC, BUN, SUA concentrations and BUN /SC ratio as comparing to control (C). The result also showed that animals treated with alcoholic extract of black currant plus either H_2O_2 (T3) or methionine (T4) showed significant decline in SC, BUN, SUA concentrations, and BUN/SC ratio with significant elevation of GSH concentration comparing to H_2O_2 (T1) and methionine (T2) treated groups. Histological section of kidney exposed to 0.5 % H_2O_2 or methionine showed acute degenerative changes characterized by vacoulation of cytoplasm of epithelial cell lining tubule with infiltration of phagocytes and monocytes, while intubation of black currant in groups T3& T4 caused regression of renal damage induced by H_2O_2 or methionine. It seems that 0.5% H_2O_2 was more effective than methionine in induction of oxidative stress and change in some biological markers related to kidney function. Also it seems that alcoholic extract of black currant exert protective actions against the damaging effect of H_2O_2 and methionine.

Keywords: Methionine overload, Kidney, H_2O_2, Black current.
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التاثير الوقائي للمستخلص الكحولي للزبيب الاسود (*Vitis Vinifera* L.) على وظيفة الكلية في ذكور الجرذان البالغة المعرضة لفرط المثيونين وبيروكسيد الهيدروجين

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الخلاصة

صممت هذه الدراسة لمعرفة تأثير زيادة المثيونين في ذكور الجرذان في أستحداث التلف الكلوي بالمقارنة مع مادة موكسدة (بيروكسيد هيدروجين). كما تهد ف هذه الدراسة الى معرفة التأثير الوقائي للمستخلص الكحولي للزبيب الأسود على بعض

المعاييرالبايلوجية المتعلقة بوظائف الكلية. تم أستخدام (50) من ذكورالجرذان البالغة قُسّمت عشوائيا الى خمس مجاميع متساوية (عشرحيوانات / مجموعة) وعوملت كألتالي لمدة 42 يوم. أعطيت الجرذان في المجموعة الاولى (C) بألتجريع الفموي محلول بفر (0.1 مولاري، PH= 7، 0.5 مليلتر) وعدت مجموعة سيطرة، في حين أعطيت المجموعة الثانية (T1) الماء الاعتيادي مضافا له بيروكسيد الهيدروجين بتركيز0.5 ٪. اما المجموعة الثالثة (T2) فقد أعطيت بألتجريع الفموي المثيونين (100ملغم / كغم / وزن الجسم) في حين أعطيت المجموعة الرابعة 0.5 (T3) ٪ من بيروكسيد الهيدروجين بماء الشرب بألاضافة الى التجريع الفموي للمستخلص الكحولي للزبيب الاسود (60ملغم / كغم / وزن الجسم) في حين جرعت حيوانات المجموعة الخامسة (T4) مزيج من المستخلص الكحولي للزبيب الاسود والمثيونين بنفس الجرع السابقة. تم اخذ عينات الدم من كل مجاميع التجربة في الايام 0، 21، 42 لغرض أجراء الفحوصات المصلية التالية : أ- قياس تركيز كلوتاثايون (GSH) (في يوم 0،42). ب- قياس تركيز الكرياتنين (SC). ج- قياس تركيز نتروجين يوريا الدم (BUN). د- قياس تركيز حامض البوليك (SUA). ي- قياس نسبة تركيز نتروجين يوريا الدم / تركيز الكرياتينين مصل الدم (BUN/SC) اضافة الى ذلك تم اخذ مقاطع نسيجية من الكلى لغرض دراسة التغيرات النسيجية المرضية. أظهرت نتائج الدراسة حدوث أنخفاض معنوي ($P<0.05$) في تركيز(GSH) في مصل دم المجموعة المعاملة ببيروكسيد الهيدروجين (T1) و المجموعة المعاملة با لمثيونين (T2) لمدة 6 اسابيع. اضافة الى حصول أرتفاع معنوي ($P<0.05$) في تركيز الكرياتنين، نتروجين يوريا الدم، وحامض البوليك و نسبة نتروجين يوريا الدم / الكرياتنين مقارنة مع مجموعة السيطرة. كما بينت النتائج ان التجريع الفموي بالمستخلص الكحولي الزبيب الاسود مضافا اليه اما بيروكسيد الهيدروجين في مجموعة (T3) اوالمثيونين في مجموعة (T4) أدت الى حدوث أنخفاض معنوي ($P<0.05$) في تركيز كل من الكرياتنين و نتروجين يوريا الدم، وحامض البوليك و نسبة نتروجين يوريا الدم / الكرياتنين في مصل الدم عند مقارنتها مع مجموعة بيروكسيد الهيدروجين (T1) و مجموعة المثيونين (T2). أظهرت نتائج الفحص النسيجي لكلى الحيوانات المعرضة لبيروكسيد الهيدروجين والمثيونين حدوث تغيرات تنكسية حادة تمثلت بتفجي هيولي الخلايا المبطنة للنبيبات الكلوية و ارتشاح الخلايا البلعمية و خلايا وحيدة النواةفي حين ادى تجريع الحيوانات بالمستخلص الكحولي للزبيب الاسود للمجاميع T3 و T4 الى أنكفاء التغيرات المرضية الكلوية التي سببها بيروكسيد الهيدروجين والمثيونين.يبدو من نتائج هذة الدراسة أن تأثير بيروكسيد الهيدروجين (0.5%) كان أقوى من المثيونين في أستحداث الاجهاد التأكسدي. وبينت التأثير الوقائي للمستخلص الكحولي للزبيب الأسود ضد الاجهاد التأكسدي المستحدث بالمثيونين و بيروكسيد الهيدروجين في بعض المعايير المتعلقة بوظائف الكلية.

Introduction

Methionine is essential in small amount in human diet, and is sold over the country as dietary supplement. The common natural sources of this amino acid are fish and meat, especially for starter chicks and broilers (1). Rice and casein offer potential novel available sources of methionine (2). In addition, National Research Council (3) recorded that the feed sources with high percentages of methionine are blood meal, crab meal, corn gluten meal and sunflower seed meal. Methionine enters the one carbon metabolic cycle through the dietary consumption breakdown. It is then converted intracellularly to S-adenosyl methionine (SAM), which is the major biological methyl donor required for numerous cellular processes, including the formation of proteins, nucleic acids, epinephrine, melatonin, phosphatidylcholine and creatine (4).

The L. form of the methionine is used extensively in human medicine for a variety of therapeutic purposes, including pH and electrolyte balance, parenteral nutrition, pharmaceutical adjuvant and other applications (5,6). The requested use for methionine in poultry production is as feed supplement. It is generally the first limiting amino acid in poultry diet (7,8) supplementation with this essential amino acid is needed for healthy and productive poultry. It increased feed conversion efficiency, thus lowering fed costs per unit of weight gain or production (9).

While its nutritionally essential methionine overload is one of many factors responsible for causing disturbance in homocysteine metabolism resulting in accumulation of homoysteine with subsequent development of hyperhomocysteinemia (10,11). Observations in many clinical and epidemiologic studies have suggested that hyperhomocysteinemia (hHCY) is an independent risk factor for a various diseased condition including coronary artery disease, congestive heart failure (12,13). It may also be relevant for dementia and Alzheimer's disease (AD) (14,15). In addition to type II diabetes (16). This study was designed to investigate the protective effects of alcoholic extract of black currant on renal function of methionine overload and H_2O_2 treated rats.

Materials and methods

Fifty male Albino Wister rats (175-250 gm) were used in this investigation. Their ages ranged between 2.5 – 3.0 months. Animals had free access to water and standard pellets diet along the experimental period. Fasting blood samples were collected at different intervals Zero, 21, 42 days of experiment. Blood were drawn via cardiac puncture technique from anesthetized rats {intramuscular injection of ketamine (90 mg/kg B.W) and xylazine (40 mg/kg B.W)}. Seventy percent of alcoholic extract of black currant was prepared according to the procedure of (17). The rats were randomly divided into five groups (10 rats/ group) and were treated daily for 42 days as follows:- 1-Group (C): was administered daily with buffer solution 0.1M, (PH 7) by oral intubations using cavage needle and served as a control group. 2-Group (T1): was subjected to ad libitum supply of drinking water containing 0.5% H_2O_2.3-Group (T2): The rats in this group were orally intubated (by gavage needle) with DL. methionine (100 mg /kg B.W) diluting in buffer 0.1M, PH 7 (18). In addition to (0.5%) H_2O_2 or methionine, animals were intubated orally (60 mg/kg B.W) with alcoholic extract of black currant (19) resembling groups T3 and T4 respectively.

Serum GSH was determined by using a modified procedure (20) utilizing Elman's reagent (DTNB), using GSH standard curve (Figure-1). Serum creatinine (SC), serum uric acid (SUA) and blood urea nitrogen (BUN) concentrations were measured using SC, SUA, BUN kits (Biolabo, France). Kidney was prepared for histological study according to (21). Data was presented as mean ± SE and analyzed by using two way analysis of variance (ANOVA) using significant level of (P<0.05). Specific group differences were determined using least significant differences (LSD) (22).

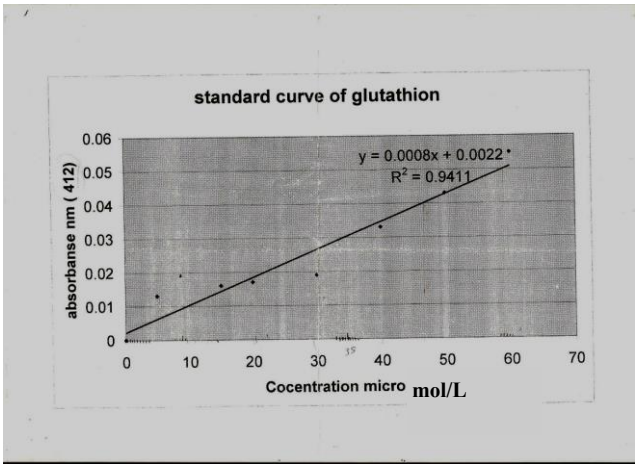

Figure 1: Standard curve of glutathione.

Results

A significant decrease (P<0.05) in the serum concentration of serum GSH concentration was observed at the end of the experimental period (after 42 day) in (T1) (7.2±0.37) and (T2) (11.2±0.49) treated groups comparing to black currant groups (T3 &T4) {(15.0±0.44), (19.0±0.31)} respectively (table 1). Highest reduction in GSH concentration was observed in H_2O_2 treated groups. Alcoholic intubations of black currant concurrently (T3or T4) caused significant increase in this parameter comparing to other treated groups. Within the time, significant decrease (P<0.05) in serum GSH concentrations were observed in the four treated groups at 42 days comparing to the values of zero day. Serum creatinine concentrations were significantly increase (P<0.05) in H_2O_2 & methionine treated groups (T1 &T2) at days 21 & 42 of the experiment comparing to black currant treated groups (T3 & T4) and control (table 2).

Within the time, significant elevation (P<0.05) in serum creatinine concentration in T1 & T2 at 42 days was observed comparing to the data at zero day with mean value of (1.10±0.08), (0.99±0.03) respectively. Significant elevation (P<0.05) in serum concentration of BUN and SUA were observed after methionine overload (T2) and H_2O_2 exposure (T1) at day 21 & 42 of the experiment comparing to the control and black currant groups. However, at the end of the experiment, alcoholic extract of black currant caused significant decrease (P<0.05) in mean value of previous parameters (table 3, 4).

Table 5 showed that intubation of methionine (T2) or exposure to 0.5% of hydrogen peroxide (T1 & T3) caused significant increment (P<0.05) in BUN/SC ratio at 21 and 42comparing to the control. On other hand, at the end of the experiment oral intubation of black currant in combination with methionine (group T4) caused significant (P<0.05) decrease in BUN/S.c ratio comparing to other treated groups (T1, T2 & T3), it seems that alcoholic extract of black currant normalize the ratio (28.28 ± 1.81) with that of control.With exception to black currant and H_2O_2 treated groups (T4), within the time, a significant increment (P<0.05) in the ratio were observed in other treated groups comparing to the pretreated period.

Histological section of rat kidney exposed to 0.5% H_2O_2 in drinking water (T1) for 6 weeks showed renal injury as indicated by acute degenerative changes including vacoulation of cytoplasm, and desquamation of epithelial cell lining of renal tubules. With infiltration of phagocytes and monocyte and mild proliferation of fibroblast (figures 3, 4) comparing to normal section of kidney (figure 2). Histological changes of rat kidney received methionine including, degeneration in the epithelial cell lining renal tubules (figure 5). While histological section of rat kidney intubated methionine plus alcoholic extract of black currant

showed mild accumulation of monocytic cell in kidney parenchyma (figures 6, 7).

Table 1: serum glutathione concentrations (micromole /L) in rats treated with 0.5% hydrogen peroxide, methionine, and alcoholic extract of black currant for 42 days.

Days	Groups				
	(C)	(T1)	(T2)	(T3)	(T4)
Zero	21. 8±0.80 A a	22.8±0.58 A a	22.4±0.68 A a	23.0±0.77 A a	22.6±0.75 A a
4 2	22.8±0.37 A a	7.2±0.37 B b	11. 2±0.49 C b	15.0±0.44 D b	19.0±0.31 E b

Values are expressed as mean ± SE, n=10 rats. C=Control ; T1,T2,T3 and T4 received 0.5% H_2O_2;100mg/Kg.B.W. methionine; H_2O_2+alcoholic extract of black currant(60 mg/kg B.W.); methionine+ alcoholic extract of black currant(60 mg/kg B.W) respectively.
Capital letters denote between groups differences, P<0.05 vs. control.
Small letters denote within group differences, P<0.05 vs. pretreated period.

Table 2: serum creatinine concentrations (mg/dl) in rats treated with 0. 5% hydrogen peroxide, methionine, and alcoholic extract of black currant for 42 days.

Days	Groups				
	(C)	(T1)	(T2)	(T3)	(T4)
Zero	0.68±0.01 A a	0.70±0.01 A a	0.69±0.01 A a	0.69±0.01 A a	0.67±0.01 A a
21	0.70±0.07 A a	1.31±0.04 B b	1.32±0.09 B b	1.06±0.06 C b	0.95±0.01 DC b
42	0.69±0.01 AC a	1.10±0.08 B c	0.99±0.03 BC c	0.87±0.02 C a	0.86±0.02 C ab

Values are expressed as mean ± SE, n=10 rats. C=Control ; T1,T2,T3 and T4 received 0.5% H_2O_2;100mg/Kg.B.W. methionine; H_2O_2+alcoholic extract of black currant(60 mg/kg B.W.); methionine+ alcoholic extract of black currant(60 mg/kg B.W) respectively.
Capital letters denote between groups differences, P<0.05 vs. control.
Small letters denote within group differences, P<0.05 vs. pretreated period.

Table 3: serum blood urea nitrogen concentrations (mg/dl) in rats treated with 0.5% hydrogen peroxide, methionine, and alcoholic extract of black currant for 42 days.

Days	Groups				
	(C)	(T1)	(T2)	(T3)	(T4)
Zero	19.3±1.2 A a	19.68±0.6 A a	19.44±0.6 A a	20.04±0.5 A a	18.60±0.4 A a
21	19.64±0.6 A a	46.0±1.6 B b	45. 2±1.1 C b	41.60±1.5 D b	40.20±1.5 D b
42	20.4±0.8 A a	41.20±1.3 B c	52.40±1.9 C c	32.68±1.4 D c	25.0±1.4 E c

Values are expressed as mean ± SE, n=10 rats. C=Control ; T1,T2,T3 and T4 received 0.5% H_2O_2;100mg/Kg.B.W. methionine; H_2O_2+alcoholic extract of black currant(60 mg/kg B.W.); methionine+ alcoholic extract of black currant(60 mg/kg B.W) respectively.
Capital letters denote between groups differences, P<0.05 vs. control.
Small letters denote within group differences, P<0.05 vs. pretreated period.

Table 4: serum uric acid concentrations (mg/dl) in rats treated with 0. 5% hydrogen peroxide, methionine, and alcoholic extract of black currant for 42 days.

Days	Groups				
	(C)	(T1)	(T2)	(T3)	(T4)
Zero	3.81±0.16 A a	3.70±0.10 A a	3.78±0.11 A a	3.70±0.15 A a	3.55±0.13 A a
21	3.89±0.14 A a	4.66±0.16 B b	4.50 ±0.19 BC b	4.30±0.21 AB c	4.18±0.14 AC b
42	3.79±0.13 A a	4.91±0.17 B b	4.71±0.14 B b	4.21±0.21 A b	4.0±0.29 A b

Values are expressed as mean ± SE, n=10 rats. C=Control ; T1,T2,T3 and T4 received 0.5% H_2O_2;100mg/Kg.B.W. methionine; H_2O_2+alcoholic extract of black currant(60 mg/kg B.W.); methionine+ alcoholic extract of black currant(60 mg/kg B.W) respectively.
Capital letters denote between groups differences, P<0.05 vs. control.
Small letters denote within group differences, P<0.05 vs. pretreated period.

Table 5: Serum Blood Urea Nitrogen/Serum Creatinine ratio in rats treated with 0.5% hydrogen peroxide, methionine, and alcoholic extract of black currant for 42 days.

Days	Groups				
	(C)	(T1)	(T2)	(T3)	(T4)
Zero	28.40±2.63 A a	27.79±0.73 A a	28.07±1.34 A a	29.05±0.85 A a	27.77±0.68 A a
21	27.93±1.08 A a	35.12±1.69 B b	34.82 ±2.39 B b	39.57±1.88 BC b	42.18±2.33 C b
42	28.86±1.05 A a	38.23±1.27 B b	53.30±3.14 C c	37.64±2.66 B b	28.28±1.81 A b

Values are expressed as mean ± SE, n=10 rats. C=Control ; T1,T2,T3 and T4 received 0.5% H_2O_2;100mg/Kg.B.W. methionine; H_2O_2+alcoholic extract of black currant(60 mg/kg B.W.); methionine+ alcoholic extract of black currant(60 mg/kg B.W) respectively.
Capital letters denote between groups differences, P<0.05 vs. control.
Small letters denote within group differences, P<0.05 vs. pretreated period.

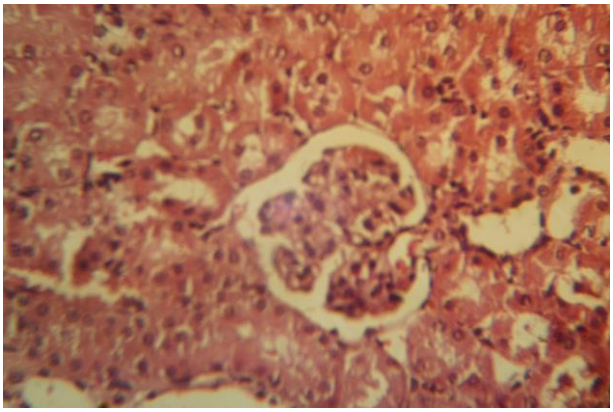

Figure 2: Histological section in kidney of control group. Note: normal histology of kidney section (40x H & E).

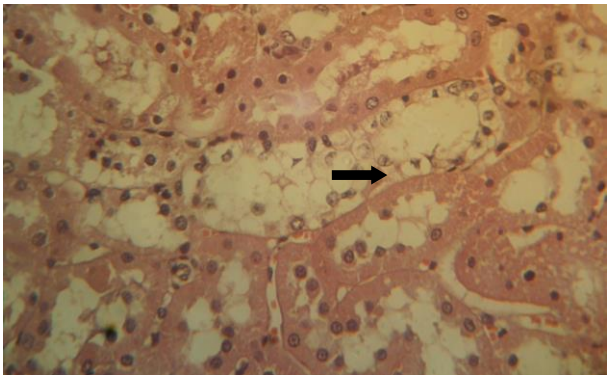

Figure 3: Histological section in kidney of H_2O_2 treated rat. Note: acute degenerative changes including, vacoulation of cytoplasm desquamation epithelial cells of renal tubule. (➡) (40x H & E).

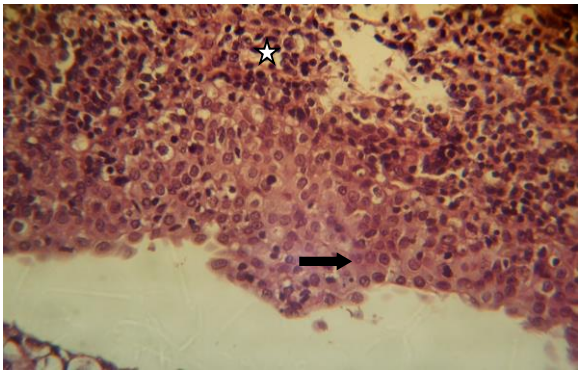

Figure 4: Histological section in kidney of H_2O_2 treated rat.Note: hyperplasia of epithelial cells lining collecting tubules (➡) and infiltration of macrophage, lymphocyte, and mild proliferation of fibroblast. (☆), (40x H&E).

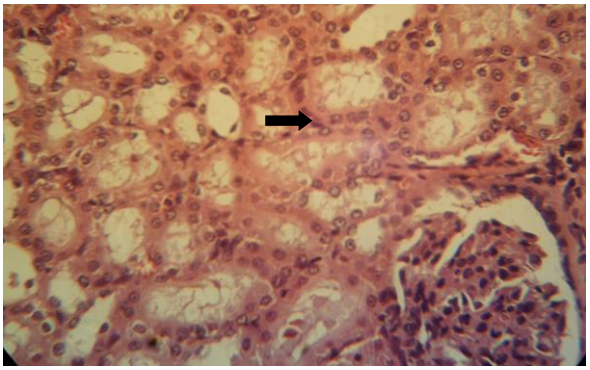

Figure 5: Histological section in kidney of methionine treated rat. Note: degeneration in epithelial cell lining the renal tubule (➡), (40x H & E).

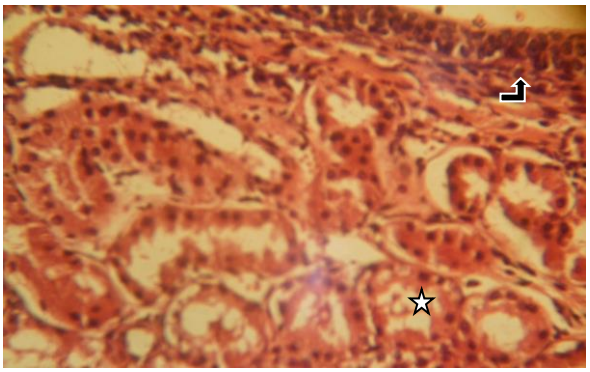

Figure 6: Histological section in kidney of H_2O_2+alcoholic extract of black currant treated rat. Note: Regression of almost all damaged tissue with exception of presence mild infiltration for monocyte cells in collecting renal tubule (⬏) and mild degeneration in epithelial cell lining the renal tubule ( ), (40x H &E).

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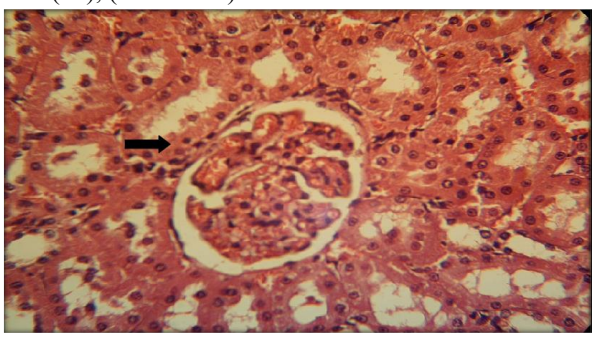

Figure 7: Histological section in kidney of methionine + alcoholic extract of black currant treated rat. Note: Regression of almost all damaged tissue with exception of mild accumulation of monocyte cells in kidney parenchyma (➡), (40x H & E).

Discussion

The results showed that oral administration of animals to 0.5% of H_2O_2 in drinking water caused pronounced decrease in serum glutathione concentration comparing to control, indicating a case of oxidative stress (23). Reduced GSH has been reported to form either nucleophil-forming conjugates with the active metabolites (ROS) or act as reductant for peroxides and free radicals produced after H_2O_2 exposure (24), which might explain its depletion in this study. On the other hand, high NO production due to various oxidative stress (like exposure to H_2O_2) can be one of the possible reasons responsible for depression in serum GSH concentration after H_2O_2 intubation (25), where formation of peroxynitrits (ONOO-) (reaction of superoxide anion and NO) strongly oxidize sulfhydral group of GSH with subsequent decrease in its concentration (26).

The role of oxidative stress as important contributing cofactors to cellular dysfunction including kidney, has substantially increased over the last years (24,27). We can hypothesized that exposure to H_2O_2 may cause elevation of superoxide anion and the dangerous hydroxyl (OH•) radical leading to glomerular dysfunction (28) with subsequent elevation in SC, BUN, SUA concentrations (29). Besides, induction of nuclear factor Kappa B ($NF\text{-}_KB$) by H_2O_2 exposure may lead to activation of a wide variety of inflammatory response like cytokines (30), thus diverse deleterious renal damage may occur with subsequent decrease in glomerular function which may result in elevation of kidney biomarkers.

A significant increase in serum BUN/SC ratio in H_2O_2 treated groups documented the renal damage which was definitely accompanied by such increase in this ratio (31).

Thus, diverse deleterious renal damage may occur. The suppression effect of methionine overload on the antioxidant status of the rats in the present study may be attributed to hyperhomocysteinemia (hHCY) induced after methionine overload (32). The result of the present work was correlated with Ventura et al.(33) and Huang et al.(34), who demonstrated significant increase in plasma markers of lipid peroxidation (LPO) in rats with hHCY resulting by induced methionine overloading. It has been demonstrated that mild hHCY is much more common and is associated with post methionine loading in water (35,36) or in diet (37). It has been recognized that methionine overload causing hHCY through disturbing remethylation pathway, preventing normal conversion of SHMT to methionine and subsequent stress of transsulfration pathway.Such hHCY will lead to formation of homocysteine S-S mixed disulfide conjugates which inhibit the superoxide radical scavenging activity of metallothionine (38,39).Accordingly, we can hypothesized that a depression in scavenging activity of metallothionine may lead to superoxide production and decrease antioxidant production including GSH in this study. The elevation of blood urea nitrogen is a positive indicator for kidney disorders especially as it relates to glomerular function (40).

Many authors indicated that high levels of blood uric acid has been correlated with gout, hypertension, renal damage, and hyperhomocysteinemia (41,42) where the proposed hyperhomocysteinemia may lead to overproduction and release of ROS from glomerulus, renal damage, impairment of glomerular filtration rate (GFR), and significant increase in creatinine clearance,serum blood urea nitrogen and creatinine concentrations (43). Furthermore, we can hypothesize that hHCY following methionine overload may produce its pathogenic effect by suppression of plasma or tissue adenosine concentration. The excess of adenosine would react with methionine forming SAM then degraded to form uric acid as its end product leading to hyperuricemia (44,45).

The results also showed significant increase BUN/SC ratio after methionine overload, which was expected as result of pronounced kidney dysfunction (29) due to methionine overload.Such ratio was reported to be increased in renal damage (31).

Concerning histological changes, hyperhomocysteinemia has been claimed to be an important causes of glomerular injury (46). Some investigators have found that hHCY induced through different approaches renal damage in both man and animals (47,48). Besides, we can hypothesized that the suspected hHCY may lead to a case of oxidative stress [as indicated by decrease in serum GSH concentration] lead to activation of PAR-4 (protease-activated receptors) (49), which induces production of ROS through increasing NADPH oxidase, decreasing thioredoxin expression and reduction nitric oxide availability.

It has been found that such decrease in NO availability after methionine overload many associated with renal injury (50). The antioxidant protective activity of grapes was documented by many investigators. Enginar et al (51) and Feng et al (52) reported that GSE inhibited lipid peroxidation and enhanced antioxidant activity in rat exposed to X-radiation.Polyphenolic compound present in grapes like resveratrol (53-55) and PCO (56) may be responsible for the antioxidant capability of the plant and has protective effect against oxidative damage induced by H_2O_2 (57) and hHcy (58). In addition, such antioxidant compound of black currant may attributed to its renoprotective effect (59-61) as indicated by suppression of BUN and SC concentrations (62). Functional differences due to black currant intubation observed in this study were also confirmed histologically, where intubation of black currant caused regression of renal lesion caused by methionine and H_2O_2 intubation. Presence of resveratrol, and all anthocyanin contents, may at least in part responsible for increased antioxidant efficiency of black currant. It has been documented that resveratrol may cause attenuation of cytochrom P-450 (63) and suppress proteinuria and hyperlipidemia all caused kidney injury (64).

In conclusion, it seems that alcoholic extract of black current exerts renoprotective action against damaging effect of H_2O_2 and methionine.

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