

EFFECT OF 1% HYDROGEN PEROXIDE (H₂O₂) IN DRINKING WATER ON SOME PARAMETERS IN ADULT MALE RABBITS

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ABSTRACT

The present study was undertaken to investigate the effect of 1% of H₂O₂ on some biochemical markers related to oxidative stress, cardiac, hepatic and thyroid functions in adult male rabbit's. Twenty adult male rabbits were divided randomly into two groups (10/group), the first (control) group and the second (T) group: animals were received 1% hydrogen peroxide (H₂O₂) in drinking water for one month. At the end of the experiment, fasting blood samples were drawn by cardiac puncture technique from all experimental animals for measuring : a- serum concentration of total cholesterol (TC); triacylglycerol (TAG); low density lipoprotein -cholesterol (LDL-C) ; very low density lipoprotein -cholesterol (VLDL-C) and high density lipoprotein -cholesterol (HDL-C) b- serum alanine transaminase (ALT) ; aspartate transaminase (AST) and lactate dehydrogenase (LD) activities c- albumin and globulin concentrations. d- Platelets count and prothrombin time e- serum glutathione (GSH) and malondialdehyde (MDA) concentrations. F- serum thyroid stimulating hormone (TSH), triiodothyronine (T₃), tetraiodothyronine (T₄) and glucose concentrations. The results revealed that male rabbits receiving 1% H₂O₂ in drinking water for one month showed a significant increase (p<0.01) in serum TC, TAG, LDL-C, VLDL-C and MDA concentrations, ALT, AST and LD activities and platelets count, and a significant decrease (p<0.05) in serum HDL-C, GSH, albumin, globulin concentrations and prothrombin time comparing to control, besides significant decrease (p<0.05) in serum T₃ & T₄ and significant increase (p<0.05) in serum TSH, and glucose concentration were observed. On conclusion, it has been found that exposure to 1% H₂O₂ exerts deleterious effect on cardiac and hepatic functions, hypothyroidism and state of oxidative stress.

Key words: 1% Hydrogen peroxide, Lipoproteins, Thyroid hormones, Liver enzymes.

تأثير 1% بيروكسيد الهيدروجين في ماء الشرب على بعض المعايير الكيموحيوية في ذكور الارانب البالغة

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الخلاصة

صممت هذه التجربة لمعرفة تأثير 1% من بيروكسيد الهيدروجين على بعض المعايير الكيموحيوية ذات العلاقة بالاجهاد التأكسدي، وظيفة القلب، الكبد والغدة الدرقية في ذكور الارانب البالغة. تم تقسيم عشرون رانباً بالغاً بصورة عشوائية الى مجموعتين متساويتين (10/مجموعة)، أعطيت المجموعة الاولى ماء الشرب العادي وهدت مجموعة سيطرة (مجموعة C). في حين أعطيت المجموعة الثانية ماء الشرب العادي الحاوي على 1% من بيروكسيد الهيدروجين لمدة شهر (مجموعة T). في نهاية التجربة تم سحب الدم من القلب مباشرة من جميع حيوانات التجربة لغرض قياس أ- تراكيز الكوليستيرول الكلي (TC)، ثلاثي أسيل الكليسيرول (TAG)، الكوليستيرول في الشحوم البروتينيه ذات الكثافة الواطئة (LDL-C)، الواطئة جداً (VLDL-C) والعالية (HDL-C) في مصل الدم؛ ب- فعالية الانزيمات الناقلة للأمين (ALT,AST) وانزيم اللاكتيت ديهيدروجينيز (LD) في مصل الدم، ت- تركيز الالبومين والكلوبيولين في مصل الدم؛ ث- قياس أع داد الصفائح الدموية وزمن تخثر البروثرومبين؛ ج- تركيز الكلوتاثايون (GSH) والمالوندايالديهيد (MDA) ح- قياس تركيز هرمونات الغدة الدرقية (T3,T4) والهرمون المحفز للدرقية (TSH) وتركيز الكلوكوز في مصل الدم. لقد أظهرت النتائج حدوث ارتفاع معنوي ($P<0.05$) في تركيز الكوليستيرول الكلي، ثلاثي أسيل الكليسيرول، الكوليستيرول في الشحوم البروتينيه ذات الكثافة الواطئة، الواطئة جداً والمالوندايالديهيد في مصل الدم فضلاً عن الارتفاع الحاصل في أعداد الصفائح الدموية وفعالية الانزيمات الناقلة للأمين (ALT,AST) وانزيم اللاكتيت ديهيدروجينيز (LD) في مصل دم ذكور الارانب البالغة بعد تعرضها ل 1% من بيروكسيد الهيدروجين في ماء الشرب لمدة شهر. كما أظهرت النتائج حدوث انخفاض معنوي ($P<0.05$) في تركيز الكوليستيرول في الشحوم البروتينيه ذات الكثافة العالية، الكلوتاثايون، الألبومين والكلوبيولين في مصل الدم مع حدوث ارتفاع معنوي في زمن تخثر البروثومبين مقارنة مع مجموعة السيطرة. بالإضافة الى ذلك أدى التعرض لبيروكسيد الهيدروجين الى حدوث انخفاض معنوي ($P<0.05$) في تركيز هرموني T3 و T4 وارتفاع معنوي في تركيز هرمون ال TSH والكلوكوز في مصل الدم. يستنتج من نتائج التجربة إن التعرض لبيروكسيد الهيدروجين بتركيز 1% قد تسبب في إحداث تأثيرات ضارة على وظيفة القلب والكبد، حالة نقص الدرقية بالإضافة الى الاجهاد التأكسدي.

INTRODUCTION

Many reactive intermediates, such as electrophiles (trends to accept electrons) and free radicals (FRs) (with the ability to damage cellular components) are produced during physiological and pathological processes. The consequences of the damage initiated by these metabolic by products affect large range of biological reactions, like increase in the mutation rate and alteration of cellular membranes composition, structural proteins (1).

Free radical(FR) production in organisms with aerobic metabolism is a continuous and unavoidable process since molecular oxygen reduction to water within the mitochondrial respiratory chain is not 100% efficient in this way(2). Mitochondria is the main source of FRs,due to electron leakage in the respiratory chain,with the resulting formation of reactive oxygen species (ROS), such as superoxide anion O_2^- , hydrogen peroxide (H_2O_2) and the more dangerous hydroxyl radicals (OH^\cdot).Other ROS producing system,such as cytochrom P450 oxidase family, NADPH oxidase and xanthine oxidase (3).

Hydrogen peroxide (H_2O_2), anon radical reactive oxygen species is generated in vivo by several enzymes system and, additionally, it is produced intracellularly by superoxide anion radical (O_2^-). In vivo, H_2O_2 is a weak oxidizing and reducing agent, killing bacteria virus and fungus (4).

H_2O_2 has been medically used as disinfectant, water purifier and bleaching agent's.It is also a common ingredient in contact lens cleaner, eye drops, mouth washes (1%) and tooth paste (5).

Although H_2O_2 is weakly reactive, its major toxicity derives from its conversions to highly toxic hydroxyl radicals (OH^\cdot) via fenton or Halser-Weiss reaction (6).Where lipid peroxidation (LPO) and the resultant oxidative stress produced by ROS including H_2O_2 are strongly implied in the pathogenesis of several disorders such as neurodegenerative disease (7), immune disease(8, 9), arteriosclerosis (10), diabetes (11) as well as cancer (12),rheumatoid arthritis and toxicity associated with aging (13).Therefore, this study was conducted to evaluate the effects of 1% of hydrogen peroxide in drinking water on hepatic, cardiac and thyroid functions in adult male rabbits.

MATERIALS AND METHODS

wenty adult male rabbits were randomly divided into two equal groups (10/group) and treated as following for one month.Group I: animals in this group were received orally ordinary tap water serving as control; animals in group (T) were orally received 1% hydrogen peroxide (H_2O_2) in ordinary tap water.

Fasting blood samples was collected from all experimental animals at the end of experiment by cardiac puncture technique for measuring some biochemical parameters related to cardiac, hepatic and thyroid functions including: serum glutathione (GSH) and malondialdehyde (MDA) concentrations according to (14) and (15) respectively; serum total cholesterol (TC), triacylglycerol (TAG);high density lipoproteins – cholesterol (HDL-c); enzymatically (Bicon –Germany), low density lipoproteins – cholesterol (LDL-c), very low density lipoproteins–cholesterol (VLDL-c)

concentrations according to (16). Prothrombine time using Prothrombine time kit (Biolabo SA, company-France), total platelet count (14), serum globulin concentration as described by (17). While serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Lactate dehydrogenase (LD) activities and serum albumin concentration using chemical kits (Randox Laboratories, UK). Serum triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone concentrations using ELFA (Ezyme Linked Flurescent Assay) technique according to (18). Serum glucose concentration was determined by enzymatic kit (Barcelona, Spain). Data were expressed in means (\pm SE) where appropriate and analyzed statistically by using t-test and those at $P < 0.05$ were accepted as significant (19).

RESULTS AND DISCUSSION

Plasma TC, LDL, VLDL and TAG concentrations was increased significantly ($p < 0.05$) after one month of treatment with H_2O_2 comparing to control. The mean values of these previous parameters were 296.4 ± 4.18 , 246.0 ± 1.59 , 43.36 ± 1.38 and 215.0 ± 6.30 for TC, LDL, VLDL and TAG respectively, comparing to the values in the control group which were 130.6 ± 2.57 , 68.04 ± 3.61 , 26.36 ± 0.19 and 131.2 ± 1.02 respectively. The mean values of cholesterol in HDL of control group was equal to (35.2 ± 1.16) , while addition of 1% H_2O_2 to drinking water for one month caused significant ($p < 0.05$) decrease in this parameter (6.8 ± 0.49) as described in table -1.

This result was similar to the findings of Khudaier (20) on rat and Nasrat (21) on Japanese Quail. Such changes in serum lipid may reflect the suppression of lipid metabolism due to H_2O_2 induced oxidative stress. Partial deficiency of lipoprotein lipase (the key enzyme determining the removal rate of TG from plasma), associated with increased output of lipoprotein from the liver may contribute to the elevation of serum TG level in H_2O_2 treated group (22). Serum HDL-C level have been reported to be inversely correlated with serum VLDL, TAG levels, both in normolipidemic and hyperlipidemic subjects (23). The present experiment confirms this statement.

Table (1) Effect of 1% hydrogen peroxide on serum lipid profile (TC, HDL-C, LDL-C, VLDL-C and TAG) concentrations of adult male rabbits. (Mean \pm SE ; n=5)

Parameters Groups	(TC) Mg/dl	HDL -C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	TAG mg/dl
Control (C)	130.6 ± 2.5	35.2 ± 1.16	69.16 ± 3.61	26.24 ± 0.19	131.2 ± 1.0
H2O2 Treated	296 ± 4.18 *	6.8 ± 0.49 *	246.06 ± 1.59 *	$43.36 \pm 1.38^*$	$215.0 \pm 6.30^*$

*Denote between groups differences, $P < 0.05$.

The result also showed that 1% H_2O_2 caused significant increase ($p < 0.05$) in serum ALT and AST activity with mean values of 98.4 ± 3.4 and 100.2 ± 2.8 for ALT and AST respectively, comparing to the values in the control (51.4 ± 1.3) and (74 ± 0.78) respectively (Table -2). The change in serum LD activity in H_2O_2 treated group showed the same trend as transaminase enzymes with mean values of (100.9 ± 2.4) comparing to the values in the control (39.98 ± 1.0).

Table(2) Effect of 1% hydrogen peroxide on serum ALT, AST and LD activities, GSH and MDA concentrations of adult male rabbits.

Mean \pm SE ; n=5					
Parameters Groups	ALT IU/L	AST IU/L	LD IU/L	GSH μ /L	MDA μ /dl
Control (C)	51.4 \pm 1.3	74 \pm 0.78	39. 98 \pm 1.0	16.6 \pm 1.33	0.41 \pm 0.02
H2O2 Treated	98.4 \pm 3.4 *	100.2 \pm 2.8*	100.9 \pm 2.4*	5.0 \pm 0.3 *	1.08 \pm 0.04*

*Denote between groups differences, $P < 0.05$.

The correlation between hepatic tissue damage and elevation of liver enzymes activities has been verified (24). It has been documented that H_2O_2 acts on stimulation of mitochondrial P450 cytochrome system of liver which play an important role on elevation of free radicals production, alteration in biological membrane with exhaustion of antioxidant enzymes leading to oxidative stress (25,26) and liver damage.

Elevation in serum ALT, AST and ALP may be a reflection of radical-mediated lipid peroxidation of liver cell membrane and impairment of enzymatic activity. Besides hyperlipidemia induced in this study may lead to development of hepatic injury (27,28) as indicated by elevation of liver enzymes.

The change in GSH and MDA after H_2O_2 were also significant when compared to the control. Significant depression in GSH and elevation in MDA concentration ($p < 0.05$) were observed with mean values of (5.0 \pm 0.3) and (1.08 \pm 0.04) respectively comparing to the values in the control group (Table-2). The oxidative effect of H_2O_2 have been documented in various diseased condition by many authors. During heavy exposure to ROS, including H_2O_2 , the level of superoxide anion and other oxidants like H_2O_2 will increase tenfold with subsequent increased demand upon the antioxidant defense system of the body including GSH leading to antioxidant depletion (29). Besides, hypercholesterolemia caused by H_2O_2 exposure in the currant experiment could be accompanied by an intensified elevation in MDA level and depression in GSH content of various tissue and blood (20,30).

The results in table (3) revealed a significant depression ($p < 0.05$) in serum albumin (0.57 \pm 0.05) and globulin (0.50 \pm 0.04) concentrations after exposure to 1% H_2O_2 in drinking water comparing to control.

Table (3) Effect of 1% hydrogen peroxide on serum albumin and globulin concentrations, Prothrombin time and Platelets count of adult male rabbits (Mean \pm SE ; n=5).

Parameters Groups	Albumin g/dl	Globulin g/dl	Prothrombin time/ second	Platelets count/mm ³
Control (C)	2.54 \pm 0.21	1.74 \pm 0.2	21.20 \pm 0.8	2.66 \pm 0.14
H ₂ O ₂ Treated	0.57 \pm 0.05*	0.50 \pm 0.04*	9.80 \pm 0.37 *	5.96 \pm 0.05 *

*Denote between groups differences.P<0.05.

Reactive oxygen species (including H₂O₂) are able to attack protein and lipids leading to membrane lipid peroxidation, and cellular dysfunction (31) free radicals produce throughout oxidative stress are able to damage the peptide back bone of protein, generated carbonylated proteins(32) with eventual formation of advanced glycation and lipid peroxidation (33). It can be perceived that this might also lead to protein miss folding –unfolding causing its depression. Sulfhydryl group of serum proteins, including serum albumin, have been suggested to be a sacrificial antioxidant in plasma and extravascular space(34), thus free radicals produced after H₂O₂ exposure may mediated oxidation, proteolysis and poor degradation of albumin leading to its depletion (35).

The result showed that daily oral intubation of H₂O₂ in drinking water for one month caused significant depression in prothrombin time (9.80 \pm 0.37) with significant elevation (p<0.05) in platelet count (5.96 \pm 0.05) after H₂O₂ intubation comparing to the control (table 3). This can be attributed to oxidative stress induced by H₂O₂ leading to enhancement of platelet aggregation & subsequently lowering prothrombin time (36). Further studies have also showed that high concentration of LDL displayed an enhanced sensitivity to thrombocyte aggregation and suppression in prothrombin time (37). Accordingly, high concentration of LDL-C in this study may be responsible for thrombocytosis and suppression of prothrombine time (38). The data explain that exposure to 1% of H₂O₂ in drinking water caused significant depression (p<0.05) in thyroid hormones T3 and T4 concentrations and a significant elevation (p<0.05) in TSH and glucose concentration as comparing to the control (Table -4).

Table (4): Effect of 1% hydrogen peroxide on serum T4, T3 and TSH concentrations of adult male rabbits.

Mean \pm SE ; n=5				
Parameters Groups	T4 (μ g/dl)	T3 (ng/dl)	TSH (μ g/dl)	Glucose (mg/dl)
Control (C)	2.55 \pm 0.09	53.37 \pm 0.97	0.74 \pm 0.02	94.93 \pm 0.52
H ₂ O ₂ Treated	1.0 \pm 0.05*	39.53 \pm 0.59*	1.53 \pm 0.13*	165.08 \pm 18.7*

*Denote between groups differences.P<0.05

In the present study hypothyroidism were observed after H₂O₂ exposure. We can supposed that H₂O₂ supplementation and the subsequent increased in H₂O₂ generation may stimulate Wolff-chaikoff effect to avoid oxidation and organification of iodide leading to depression of T₃ and T₄ formation (39). Besides, the case hypothyroidism in this study may be due to the effect of H₂O₂ on deiodination process which are mediated by peroxidase-hydrogen peroxide system (40). Where *in vitro* H₂O₂ intubation caused significant increased in type 3 deiodinase (D3) activity causing degradation of T₃ and T₄ with subsequent depression of both hormones (41), as well as the suspected deficiency of selenium due to oxidative stress may play a role (42).

Serum glucose concentration was significantly decrease in hydrogen peroxide treated animals indicating that H₂O₂ impaired insulin action effectively leading to hyperglycemia. Besides, oxidative stress induced by H₂O₂ was associated with development of insulin resistance, stimulation of gluconeogenesis and the resultant hyperglycemia (43,44).

In conclusion, it is plausible to suggest that H₂O₂ may trigger the production of ROS coupled with impaired oxidant/antioxidant balance, and hyperlipidemia leading to hepatic and cardiac damage, hyperglycemia and state of hypothyroidism.

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