RESEARCH ARTICLE

Effect of Anti-oxidant Activity of Kiwi Fruit Against Oxidative Stress Induced by Hydrogen Peroxide in Male Mice

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ABSTRACT

Kiwi fruit is among the most valued fruits in the world due to its flavor and health advantages. This study is carried out to assess the anti-oxidant and beneficial effect of kiwi fruit on some biochemical and blood parameters in male mice exposed to oxidative stress. Thirty (30) male mice were randomly distributed into three groups (group contained ten mice). The first group was considered as a control group (C), second group T1 [0.75% hydrogen peroxide (H_2O_2)] received in drinking water for 30 days, and the third group T2 treated with oral administration with 250 mg/kg aqueous extract of kiwi fruit and received H_2O_2 (0.75%) in drinking water daily for 30 days. The results clarified that male mice received H_2O_2 induced oxidative stress causes significant ($p < 0.05$) elevation alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, and bilirubin, while a reduction in glucose level and total protein on biochemical parameters. In addition, it has a deleterious effect on hematological parameters through reduction of red blood corpuscle (RBC), hemoglobin (Hb), hematocrit, mean corpuscular hemoglobin (MCH), and white blood cell (WBC). The result clarified the anti-oxidant effect of kiwi fruit in group T2 manifested by enhancement biochemical and hematological parameters compared to T1. It could be concluded that kiwi fruit possessed anti-oxidant and protective activity on a biochemical and hematological parameter of the oxidative stressed male mice.

Keywords: Anti-oxidants, Biochemical, Hematological, Hydrogen Peroxide, Kiwifruit.

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INTRODUCTION

Oxidative stress is a disturbance in the balance between anti-oxidants and oxidants levels.[1] Reactive oxygen species (ROS) are free radicals, highly reactive molecules, or atoms which the main causes of oxidative stress. These ROS cause cellular damage through oxygen interaction with certain molecules, then cellular macromolecules act as oxidants or reductants by accepting or losing electrons subsequently, serial reactions damage DNA, lipids and proteins.[2] Recent study reported that hydrogen peroxide (H_2O_2) act as oxidative stress inducer in animal models.[3] Eating habits and modern lifestyle are considered one of the main causes of oxidative stress inducers.[4] So, anti-oxidant foods such as vegetables and fruits, particularly Kiwi fruits, have important health roles for healthy as protective features against diseases resulting from oxidative stress.[5]

Kiwifruit (*Actinidia deliciosa*) belongs to the family *Actinidiaceae*. Kiwifruit is a rich nutrient fruit, and regular consumption improves the immune, digestive, and metabolic system.[6] Therapeutic features of Kiwi fruit belongs to are extremely high in vitamin C and has a range of other nutrients: including vitamin E, dietary fiber, folate, and potassium.[7] Kiwifruit consumption is frequently believed in anti-diabetes, obesity, and cardiovascular disease prevention.[8] Surely, Kiwi fruit has been shown to have a variety of biological functions, including anti-inflammatory, anti-oxidant, and anti-proliferative properties.[9-11] The anti-oxidant activity of Kiwi fruits was the ability to reduce oxidative and DNA damage.[12,13] *In vitro* study has shown that kiwi fruit extracts prevent tumor development.[14]

The current study was carried out as a therapeutic way to determine the anti-oxidant activity of kiwi fruit to reduce the harmful effects of oxidative stress caused by H_2O_2 on biochemical and hematological parameters in male mice. The findings of this study may provide useful knowledge for our society, which supports kiwi fruit as an important nutrient in daily life.

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MATERIAL AND METHODS

Preparation of Kiwi Fruit

Fresh Iraqi kiwifruits were obtained from a market in (Al-fajer town, Thi-Qar city) and thoroughly cleansed with water and separated from the peeling skin. Cut into small pieces and dry. Once the fruits had dried completely, they were powdered, and 100 g of ground fruit was soaked in 2.5 L of distilled water before being shaken for ten hours using a mechanical shaker. The extract (combination) had been sieved through a cotton or glass wool sieve. The filtrates were evaporated using a rotavapor at temperatures ranging from 40 to 45°C and obtained extracts at 0 to 4ºC storage until use.[15] A known amount of the leftover extracts was diluted with water and given to the mice orally.

Experimental Animals

Thirty mature male mice, aged 12–16 weeks (starting 25–30 g weight), were obtained from an animal house in Science College, Thi-Qar University for the experiment. They were accommodated in a quiet room in plastic cages with sawdust and allowed free access to water and pelleted food. The room temperature was kept at 24 ± 1°C, and lights were turned on at 6:00 AM and turned off at 6:00 PM (12:12 hours light: dark cycle). The University of Sumer Ethics Review Committee for Animals Experimentation accepted the experimental protocol.

Experimental Design

The male mice were distributed into three treated groups at random, with each group consisting of ten mice.

- Control Group: Ten male mice drank water with no additions for 30 days.
- T1 treated group: Ten male mice drank 0.75% H_2O_2 in drinking water daily for 30 days.[16]
- T2 treated group: Ten male mice drank 0.75% H_2O_2 in drinking water and were daily given an aqueous extract of Kiwi fruit (250 mg/Kg) orally for 30 days.

Blood Samples Collection

The CLSI guideline H21-A5 was used to authorize sample collection and animal handling.[17] The animals were anesthetized with chloroform. Blood was drawn straight through cardiac puncture using syringes and needles at the end of the experiment (day 31). blood samples (1-mL) were divided into two parts: one was retained in EDTA tubes for hematological characteristics measurement (RBC, WBC, Hb, PCV, MCH, MCV, MCHC, and PLT), while the other was kept in normal labeled test tubes for serum separation. The serum was sent to a lab to analyze biochemical parameters (levels of ALT, ALP, AST, ALP, glucose, urea, creatinine, bilirubin, and total protein).

Investigation of the Hematology

The blood parameters were determined using a complete hematology analyzer (Al-Rahma for hematological and biochemical analysis). On EDTA anticoagulated blood, this hematological analyzer performed a full blood count (CBC).[18]

Biochemical Parameters

Total protein, AST, ALT, ALP, and Bilirubin were assessed for the liver function evaluation, and urea and creatinine were measured for the kidney function assessment. A UV visible spectrophotometer was used to determine these biochemical parameters using the spectrophotometric approach. A glucometer (test strips) was used to measure glucose levels (Contour, Japan). The Biuret method was used to determine total protein.[19] The colorimetric assay for measurement hydrazone produced with 2,4-dinitrophenyl hydrazine was used to detect alanine and aspartate aminotransferases.[20] The phenolphthalein monophosphate technique was used to determine alkaline phosphatase.[21] Bilirubin was determined according to Jendrassik method.[22] Creatinine was determined based on Jaffe's method.[23] Serum urea was measured according to Rock *et al*.[24]

Statistical Analysis

The data were presented as a mean and SE. The data was analyzed using statistical software (IBM SPSS Statistics 21). One-way ANOVA followed by Duncan post hoc test for between-groups comparisons using the LSD. Statistical significance was defined as a value of $p < 0.05$.

RESULTS

As shown in Table 1, effect of H_2O_2 and aqueous extract of kiwi fruit on liver function were no signs in AST, ALT, ALP, TP, and bilirubin in group T2, which exposed to H_2O_2 and aqueous extract of kiwi fruit with mean values of (61.00 ± 3.06), (49.80 ± 1.77), (48.40 ± 2.11), (5.78 ± 0.35) and (0.32 ± 0.02) respectively as compared with values of a control group which was (52.60 ± 3.20), (45.26 ± 2.99), (43.40 ± 1.36), (6.20 ± 0.35) and (0.26 ± 0.01) respectively. While, T1 group, which was exposed to H_2O_2 showed a significant increase ($p < 0.05$) in this parameter except TP compared to group C and group T2.

The results in Table 2 showed a significantly increase in kidney function marker (urea and creatinine) and glucose in group T1 as compared with group C (35.00 ± 1.67 *vs*. 59.80 ± 2.37), (0.70 ± 0.01 *vs*. 2.10 ± 0.20) and (102.80 ± 1.49 *vs*. 187.20 ± 5.86) respectively. Whereas, T2 group showed no significant difference in this parameter except urea as comparing to group C. Also, a significant decrease ($p < 0.05$) in this parameters in group T2 as comparing with group T1.

Table 1: Effect of H_2O_2 and aqueous extract of kiwi fruit on hepatic function

	*Mean ± SE**		
Parameters	*C*	*T1*	*T2*
ALT U/L	52.60 ± 3.20 b	104.20 ± 4.39 a	61.00 ± 3.06 b
AST U/L	45.26 ± 2.99 b	83.40 ± 5.08 a	49.80 ± 1.77 b
ALP U/L	43.40 ± 1.36 b	73.60 ± 3.65 a	48.40 ± 2.11 b
TP gm/dL	6.20 ± 0.35 a	4.26 ± 0.17 b	5.78 ± 0.35 a
Bilirubin gm/dL	0.26 ± 0.01 b	0.59 ± 0.03 a	0.32 ± 0.02 b

Significant differences in $p < 0.05$ between groups are meant by different letters in the same row.

The obtained results in Table 3 clarified a significant reduction ($p < 0.05$) in hematocrit, RBC, Hb, MCHC, while an increase in MCV and PLT values in group T1, which exposed to H_2O_2 in water as comparing with group C. On the other hand, an animal exposed to H_2O_2 and aqueous extract of kiwifruits showed elevation of these parameters compared with the T1 group, which drank H_2O_2 in water.

Our results about WBCs count and WBCs differential count in Table 4 revealed that group T1 which exposed to H_2O_2 appeared significant ($p < 0.05$) decreased in WBCs and lymphocyte and increased in monocytes as comparing to the group C. Results also indicated that co-administration of H_2O_2 and aqueous extract of kiwifruits significantly $p < 0.05$ increase of WBC as comparing with group treated with H_2O_2.

DISCUSSION

H_2O_2, which is one of the common ROS, induces oxidative stress in an animal model; the present result indicated exposed to H_2O_2 in water induces hepatic injury and necrosis as observed by the increase hepatic enzyme (AST, ALT, and ALP), bilirubin, urea, creatinine, glucose, and decrease in total protein in group T1. H_2O_2 induces oxidative stress, which led to hepatotoxicity and nephrotoxicity.[16] Increase of these enzymes in blood result from rupture of the plasma membrane of hepatic cell and cellular injury led to release these enzymes.[25] Therefore, they were released into the blood following cellular injury; serum ALT and AST were indicators in the diagnosis of liver damage.[26] Also, the increase in liver enzymes could be due to radical-mediated lipid peroxidation of the hepatic cell membrane. It's maybe H_2O_2 causes oxidative stress by depleting liver glutathione and binding to liver cell and mitochondrial protein, resulting in necrosis and enhanced lipid peroxidation in liver tissue.[27]

The present study demonstrated that aqueous extract of kiwifruits in group T2 have enhanced effect against nephrotoxicity and hepatotoxicity which induced by H_2O_2, our result revealed a decrease in hepatic enzymes ALT, AST, ALP, bilirubin, urea and creatinine in addition elevation total protein group T2 (H_2O_2 plus kiwi fruit) as comparing with group T1 (H_2O_2) may be due to anti-oxidant compound in an extract of kiwi fruit which act as block free radicals which induced lipid peroxidation result from H_2O_2. Phytochemical analysis of kiwifruit revealed present naringin, ferulic acid, gallic acid, rutin, caffeic acid, protocatechuic acid, syringic acid, catechin, and salicylic acid.[28] therefor, these phytochemical compounds act anti-oxidant properties for scavengers free radicals.[29]

The our results indicated that treatment with H_2O_2 causes harmful effect on hematological traits. As it generates oxidative stress owing to the release of HO^-, which has a negative impact on plasma membranes of RBC, causing aging, deformities, and eventually cell death.[30,31] Furthermore, free radicals react with the heme molecule, lowering its content in RBCs.[32] Also, H_2O_2 induced hepatotoxicity and impaired protein synthesis and reduced total serum protein, consequently protein synthesis deficiency which largely leads to decreased essential amino acids and lack of protein

Table 2: Effect of H_2O_2 and aqueous extract of kiwi fruit on some biochemical parameters

	*Mean ± SE**		
Parameters	*C*	*T1*	*T2*
Urea gm/dL	35.00 ± 1.67 c	59.80 ± 2.37 a	41.20 ± 1.24 b
Creatinine gm/dL	0.70 ± 0.01 b	2.10 ± 0.20 a	0.94 ± 0.07 b
Glucose gm/dL	102.80 ± 1.49 b	187.20 ± 5.86 a	113.40 ± 4.41 b

Significant differences in $p < 0.05$ between groups are meant by different letters in the same row.

Table 3: Effect of H_2O_2 and aqueous extract of kiwi fruit on blood parameters

	*Mean ± SE**		
Parameters	*C*	*T1*	*T2*
RBC × 10^6/mL	7.06 ± 0.40 a	5.12 ± 0.32 b	6.64 ± 0.34 a
Hb gm/dL	13.28 ± 0.47 a	9.18 ± 0.22 c	11.96 ± 0.41b
PCV (%)	42.00 ± 1.14 a	33.60 ± 1.46 b	40.00 ± 0.70 a
MCV (fl)	59.62 ± 2.09 b	68.51 ± 2.08 a	60.57 ± 1.73 b
MCH (Pg)	18.62 ± 0.624 a	18.79 ± 0.81 a	18.59 ± 0.55 a
MCHC (%)	31.08 ± 0.40 a	27.41 ± 0.62 b	30.78 ± 1.20 a
PLT × 10^3/mL	295.40 ± 26.66 b	540.80 ± 40.86 a	359.40 ± 16.35 b

Significant differences in $p < 0.05$ between groups are meant by different letters in the same row.

Table 4: Effect of H_2O_2 and aqueous extract of kiwifruit WBC and differential WBC count

	*Mean ± SE**		
Parameters	*C*	*T1*	T2
WBC 10^3/mm^3	6.70 ± 0.21 a	4.60 ± 0.25 c	5.86 ± 0.17 b
Lymphocyte s (%)	71.50 ± 1.58 a	65.00 ±1.61 b	69.00 ± 1.51 ab
Monocytes (%)	16.16 ± 0.41b	21.40 ± 0.97 a	17.40 ± 0.87 b
Granulocytes (%)	11.76 ± 0.94 a	13.60 ± 0.81 a	13.60 ± 0.67 a

Significant differences in $p < 0.05$ between groups are meant by different letters in the same row.

synthesis sources of electricity integrated into the formation of hemoglobin and anemia.[33] The number of platelets in mice treated with H_2O_2 is rising due to the increased number and activity of platelets due to free radicals.[34] While kiwi fruit contain phytochemical substances include polyphenol, flavonoid, vitamin E, tocopherol, chlorogenic acid, sterols, ursolic acid, caffeic acid, triterpene, coumarin, glucosides, β-sitosterol, and stigmasterol.[29,35] These phytochemicals are well-known hemopoietic agents that directly affect blood production, and anti-oxidant activity leads to inhibitory free radicals and avoid hemolytic anemia.[36] This result agrees with Azab 2021, who reported kiwi fruit has hemopoietic activity by enhancing the RBC count and Hb, which decrease by oxidative stress.[37]

In the present study, chronic exposure to H_2O_2 induced a significant decrease in total WBCs and lymphocytes and an increase in monocytes resulting from oxidative stress induced by H_2O_2.[16] This effect caused a reduction WBCs count. This decrease in WBC and lymphocyte count could be related to WBC DNA and cell membrane damage; also, oxidative stress increases cortisol release, which is considered immunosuppressive.[38] Our result agrees with Khudair (in 2008), who reported exposure to H_2O_2 causes leucopenia and lymphocytopenia.[39] Co-administration of kiwi fruit and H_2O_2 shown increase WBC compared with group H2O2 and return WBC differential count near to normal value due to kiwi fruit containing anti-oxidant compounds such as the content of natural anti-oxidants such as saponins, alkaloids, cardiac glycosides terpenoids, tannins, vitamin E, C and flavonoid.[40] Vitamin C aids immune protection by supporting both the adaptive and innate immune systems' cellular functioning. Vit C enhances oxidant scavenging and improves epithelial barrier function against infections. Vit C accumulated in the phagocytic cell like neutrophil, where it can boost chemotaxis and phagocytosis. Vit C has been demonstrated to improve cell differentiation and proliferation in lymphocytes.[41]

CONCLUSION

Kiwi fruits are a strong source of anti-oxidants that can be employed at various doses as safe, cheap, and acceptable eating. For the many hematological and biochemical characteristics covered by this investigation, this has demonstrated a significant protective impact.

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