Immune boosting and Anti-oxidant activities of kiwifruit extract on hydrogen peroxide induced toxicity in Rats.

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ABSTRACT

Kiwi fruit is known as Chinese gooseberry and considered as natural dietary supplement with many health benefits. This study aims to assess the immune boosting and antioxidant effect of kiwi fruit (KWF) with three different forms in hydrogen peroxide (H_2O_2) induced toxicity in rats. Twenty-five rats, divided into five groups (5 rats for each); as normal control group (-ve), H_2O_2 (+ve) (100mg H_2O_2/kg bwt/day), treated groups were ((+ve) with oral KWF juice (100 mg /kg/bwt/day), ((+ve) with KWF powder (10g/kg diet/day), and ((+ve) with KWF mixture oral (100mg/kg bwt/day & powder10g /kg diet/day). At the end of the experiment, all rats were sacrificed and their serum was analyzed. The results showed that all groups treated by kiwi fruit significantly diminish the toxicity of H_2O_2 by decreasing MDA, AST, ALT, creatinine, urea and uric acid and increasing the levels of GSH, SOD, total antioxidants, blood parameters and nutritional parameters in serum of all groups which received kiwi fruit in comparison with H_2O_2 (+ve). In conclusion kiwi fruit juice and powder with different levels has some significant immune boosting and antioxidant effects.

Key words: kiwifruit, Immune, Antioxidants, Phytochemical, Anti-inflammatory

INTRODUCTION

For the time being many studies were carried to create a new approach in improving the general health naturally, it recommend that daily consumption or regularly of fruits with high bioactivity nutrients could associated with disease prevention and act as immune boosting agents (**Iwasawa *et al.*, 2011**).

The Chinese gooseberry is well known as kiwi fruit which belongs to genus *Actinidia.* There are many species belongs to that genus, the most consumed one is well known as green kiwi fruit which known as *Actinidia deliciosa* and the other one is the gold kiwi fruit(*Actinidia chinensis*) (**Ferguson and Ferguson 2003**). In the century China, kiwifruit was recorded description for the first time, which needs temperate climates to be grown such as eastern China, Chile, France and New Zealand (Kaur ***et al.*, 2010**). Kiwifruit is a large oval-shaped fruit which has a sweet and sour taste with green flesh inside, people used to consume kiwi fruit since long time ago. In folk medicine and Chinese medicine, it was given for women and children as to help them in growth and was given after delivery in order to gain power again for women. Traditionally, it has been used to treat gastrointestinal problem such as indigestion (**Beever and Hopkirk 1990**). Studies were carried out to investigate the possible health effects of kiwi fruit, especially after its chemical composition became well known. Kiwi is a good source of minerals, vitamins and phytochemical substances which makes it a good candidate to provide health benefits (**Ferguson and Bollard 1990**). Kiwifruit also contain vitamins E, folate, potassium, copper and magnesium, kiwifruit contains high amount of vitamin c which can provide the daily recommended intake of vit c for human body (**Schmeda and Yesilada, 2005**).

Carotenoids and polyphenols are major compounds of kiwifruit' phytochemicals which play a great role against DNA damage resulted from oxidative stress also can prevent or suppress cancer cell growth **(Larocea *et al.*, 2010**).

Recently many in vivo studies approved that kiwi fruit has cardiovascular protective effects besides a powerful antioxidant effects due to it contains lutein and vitamin c, plus it shows an

anti-inflammatory properties (**Park *et al.*, 2012**). Above all, kiwi has been linked to decrease plasma lipids with potential to prevent clots formation, kiwi fruit is under investigation for boosting immunity system (**Cassano *et al.*, 2006**).

Hydrogen peroxide or Oxygenated water with chemical formula H_2O_2, which is a chemical compound commercially used as an oxidizer / bleaching agent, and antiseptic (**International Agency for the Research on Cancer (IARC) 1999**). Hydrogen peroxide is unstable agent which decomposed slowly in the presence of light; H_2O_2 has main toxic effect which is irritation at the site of contact although it may cause toxicity from all routes of exposure (**International Programme on Chemical Safety (IPCS) 1998**). Pulmonary edema may occur and consider critical if route of toxicity was via inhalation (**Hill 2019**). Ingestion of hydrogen peroxide results in gastrointestinal irritation, vomiting and hematemesis in severe cases, can result in death (**International Programme on Chemical Safety (IPCS) 2000**).

Long-term oral administration of 0.1 - 0.15% hydrogen peroxide to mice gave rise to an inflammatory response in the gastro-duodenal tissue besides Hydrogen peroxide has the potential for mutagenicity in in vitro systems (**Housecroft and Sharpe 2005**). The aim of this study is to investigate the potential effect of kiwi fruit as immune boosting agent and reversing the toxicity resulted from H_2O_2 administration.

MATERIAL AND METHODS

Materials:-

Kiwifruit was obtained from local market, Mansura, Egypt. Casein, vitamins, minerals and cellulose were obtained from El-Gomhariya Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt. While starch and corn oil were obtained from local market.

- Hydrogen peroxide H_2O_2 was purchased from United Company for Chemicals and Medical Instruments and N-Acetyl was purchased from SEDICO drug industrial company.

- Twenty-five mature male albino rats of Sprague - Dawley strain weighing 130±5 g. at age of 9-12 weeks were obtained from Laboratory of Animal Colony, Helwan, Egypt.

Methods:

Fresh kiwifruit **(KWF)** were washed with water and cut into small pieces and dried by the hybrid solar convective drying system, belonging to the Solar Energy Dept., National Research Center, Dokki, Egypt, at (40-60°C) for approximately 6 – 7 hours (**A.O.A.C. 2005**).

Chemical analyses of kiwifruit powder: Fiber, moisture, and ash of kiwifruit were determined according to the methods of the (**A.O.A.C., 1995**).

HPLC analysis of polyphenols: analysis was performed using a waters 2487 HPLC system consisting of a dual λ detector and a Waters 1525 binary pump,and equipped with a Waters Symmetry® C_{18} column (5 mm, 4.6 × 50 mm) with Waters Sentry universal guard column (5 mm, 4.6 × 20 mm) (Waters Corporation, Milford, MA, USA). Phenolic compounds of kiwifruit juice were studied using the reference HPLC method by comparing experimental retention times with reported reference values **(Sakakibara *et al.*, 2003).**

Experimental design:

The rats were housed in stainless steel cages with wire mesh bottoms and maintained in temperature and humidity control with 12 hrs light / dark cycle. All rats were allowed to free access drinking of water and basal diet for seven days adjustment to the laboratory environment. Then rats were randomly divided into 5 groups (each of 5 rats) as follow:

Group (1): Negative control (v-); rats fed on basal diet only.

Group (2): Positive control (v+) were rats fed on basal diet and gave 100mg H_2O_2 /kg/bwt/rat/day in drinking water according to **Fatima *et al.*, (2012)**

Groups (3): Positive control (v+) with oral KWF juice (100 mg/ kg bwt /day).

Groups (4): Positive control (v+) with KWF powder (10 g / kg diet/day).

Groups (5): Positive control (v+) with KWF mixture oral juice (100mg/ kg bwt/ day) & powder (10g /kg diet/day).

Biochemical analysis:

Blood hemoglobin and Hematocrit (HCM) were blood picture: Blood samples analyses for red blood cells (RBCs) and platelet cell (PLT) according to **(Drabkin, 1949 and Mc-Inory 1954).**

Determination of kidney functions: Serum creatinine and urea were determined according to the methods described by **(Bartles, 1972** and **Patton and Crouch, 1977)**.

Determination of liver functions: Serum alanine, aspartate aminotransferases (ALT & AST), and alkaline phosphates (AP) enzymes were estimated according to (**Reitman and Frankel, 1957, Kind and King 1954 and Doumas *et al.*, 1973)** respectively**.**

Serum of biochemical analysis: Immediately after weighing the genitalia, each testis was homogenised for the biochemical analysis of antioxidant enzymes, including superoxide dismutase activity (SOD) , glutathione activity (GSH) were determined according to (**Nishikimi *et al.*, 1972 and Beutler *et al.*, 1963**).

Statistical an*alysis: The obtained data were statistically analyzed using computerized SPSS. Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups **(Snedecor and Cochran, 1967).**

RESULTS AND DISCUSSION

1. The Chemical composition of fresh kiwifruit.

Table (1) shows the chemical composition of kiwifruit as it contains 73.28 g/100g of moisture, 23.55g /100g of carbohydrates, 0.34 g/100g of ash, 0.05 g/100g of fat, 0.98 g/100g of protein and 1.80 g/100g of dietary fiber. There is a similarity between this result and (**Morton, 1987**), who reported in his study that kiwifruit contains moisture, protein, fat, ash and carbohydrate

(81.2 g, 0.79 g, 0.07 g, 045 g and 17.5) respectively. Also, composition of sun-gold kiwifruit reported from the USDA Food Composition Database were moisture, protein, fat, fiber and carbohydrate (82.44, 1.02 g, 0.28 g, 1.4 g and 15.79) respectively **(United States Department of Agriculture, USDA Food Composition Databases, 2018)**.

Table (1): Chemical composition of fresh kiwifruit

Nutrients	Composition (g/100g)
Moisture	73.28
Protein	0.98
Fat	0.05
Ash	0.34
Dietary fiber	1.80
Carbohydrate	23.55

Values are the means of 3 independent determinations.

2. Phenolic compounds present in kiwifruit juice.

Results in table (2), represents the phenolic compounds in kiwifruit's active constituents. The antioxidant phenolic compounds as, flavonoids, polyphenols and flavones were investigated in kiwifruit juice. The data in Table **(2)** indicate that the juice is a rich source of natural antioxidants. **Jin *et al.*, (2019)** showed that the total phenolics, flavonoids and antioxidant capacity of kiwis (16 minutes) were significantly higher by 108.65%, 105.56% and 65.67%, respectively. Which indicates that high-intensity ultrasound has tremendous potential to increase these bioactive molecules and antioxidant capacity of kiwi fruit.

Table(2): Phenolic compounds present in kiwifruit juice

Phenolic compounds	λ^a (nm)	EtR^b (min)	RtR^c (min)
Flavonoids	320	48.4	43.6
Polyphenols	290	09.1	07.5
Flavones	340	35.1	39.5

[a] wavelength for determination, [b] experimental retention time, [c] standard retention time.

3. *Effect of kiwifruit treatment on nutritional parameters on rats intoxicated by H_2O_2.*

Table (3) shows the results of nutritional parameters such as weight gain, feed intake and FER. Administration of H_2O_2 lead to decrease in weight gain, feed intake and FER of experimental rats in compared to negative control group. On the other hand, rat groups treated with kiwifruit showed a significant increase in all nutritional parameters compared to positive control group. Hydrogen peroxide is a chemical compound, may cause toxicity from all routes of exposure, Ingestion of hydrogen peroxide results in gastrointestinal irritation, abdominal pain, gastric distension and haematemesis (**Greene *et al.*, 2019**).

These results were matched with (**Lee *et al.*, 2012**) as consumption of kiwifruit improves the digestion by different mechanism through facilitating smooth traffic inside gastrointestinal tract. Kiwifruit helps in the digestion of proteins and accelerate it because it contains a high amount of actinidin which is consider as proteolytic enzyme (**Chaurasia and Gaba 2014**) another study (**Leontowicz *et al.*, 2016**) supported these results as it found that adding of kiwi fruit powder shown to help in the growth of good flora of the stomach beside it prevent anemia by improving the absorption of iron.

Table (3): Effect of kiwifruit treatment on nutritional parameters on rats intoxicated by rats H_2O_2:

Groups	Parameters		
	Weight gain (g)	**Feed intake (g/day)**	**FER**
G1 (–ve)	101.76±8.11 a	17.94± 2.20 a	0. 097±0.03a
G2(+ve)	55.64±8.11d	13.94± 2.20d	0. 063±0.06d
G 3 (KWF juice 100 mg / kg/bwt/day)	83.59± 6.11 c	16.80± 2.03 a	0.085±0.02 c
G 4 (KWF powder 10 g / kg diet/day)	85.13±9.13 c	16.66±2.32 a	0.083±0.04c
G 5 (Mixture of oral 100mg / kg/bwt/day & powder 10g /kg diet/day)	93.44±9.17 b	17.68±2.92 a	0.091±0.04 b

4- Effect of kiwifruit treatment on blood parameters on rats intoxicated by rats H_2O_2.

Each value represent the mean of three replicates ±SD. Values in each column having different superscript are significant different at p≤0.05.

Results in table (4) showed that administration of H_2O_2 decreased the value of Hg, RBCs, HCT and PLT in compare to control negative group. The hemoglobin (Hg) value of positive control group was 5.30 meanwhile it was 15.20 for negative control group. The treatment with kiwifruit reversed the effect of H_2O_2, as there was a significant increase in levels of Hg, RBCs, HCT and PLT in compare to control positive group. The best result was recorded for kiwifruit mixture oral 100mg / kg among the other kiwifruit treated groups.

Kiwifruit is good source of dietary folate (**Ferguson and Ferguson 2003**), which is play a great role in generation of healthy red blood cells. These results in parallel with (**Beck *et al.*, 2010**) who found that regular daily intake of kiwi fruit in long-term study lead to increase in red blood cell folate content and maintain hemoglobin level in normal range. Kiwi fruit is rich in a phytochemical substance called Lutein and Catechin, both of them reduce the free radicals and improve immune system by stimulating the bone marrow proliferation (**Keith, 2012**). In fact, green kiwi was showed a marked immunopotentiating effects by increasing recruiting neutrophils and the leukocyte count more than those of gold kiwi (**Lucas *et al.*, 2005**).

Table (4): Effect of kiwifruit treatment on blood parameters on rats intoxicated by rats H_2O_2

Groups	Parameters			
	HG (mg/dl)	**RBCs** *($x10^6$/ µl)*	**HCT** %	**PLT** *($x10^6$/ µl)*
G1 (–ve)	15.20± 2.01 a	5.15± 0.31 a	39.40± 5.91 a	225.00± 20.41 b
G2(+ve)	5.30± 2.85 a	2.84± 0.31 d	8.20± 6.47 d	106.00± 15.84 d
G 3 (KWF juice 100 mg / kg bwt/ day)	12.00± 2.00 c	3.42± 0.06 c	30.80± 6.19 c	161.88± 15.98 c
G 4 (KWF powder 10 g / kg diet/day)	14.50± 2.04 b	4.75± 0.15 b	32.70± 5.56 c	190.00± 41.16 bc
G 5 (Mixture of oral 100mg /kg bwt/ day & powder 10g /kg diet/day)	15.06± 1.52 b	4.98± 0.52 b	36.20± 6.52 b	204.00± 32.57 b

Each value represent the mean of three replicates ±SD. Values in each column having different superscript are significant different at p≤0.05.

5- Effect of kiwifruit treatment on liver and kidney parameters on rats intoxicated by rats H_2O_2.

The immune-booster effect of the three different forms of kiwifruit on H_2O_2-induced toxicity in rats is shown in Table 5. In normal control rats, serum uric acid levels were 1.53 ± 0.78 mg/dl. However in positive group (+ve), serum uric acid levels were elevated significantly to 3.20 ±0.52 mg/dl ($p < 0.05$). moreover, positive control group showed an increasing in value of AST, ALT, ALP, creatinine and urea in compare to negative control group (192.57, 142.86, 164.39, 5.62 and 39.93) respectively, rats treated with kiwi fruit were showed a significantly decreasing in value of AST, ALT, ALP, uric acid, creatinine and urea in compare to positive control group. During the treatment period with the kiwi fruit especially group treated with mixture oral 100mg / kg, renal function and liver function profiles gradually improved, but not uniformly (Table 5). These results are in parallel with those obtained by (**Latocha *et al.*, 2010**).

In other study, extracts of kiwi fruit species were effective and reversed the liver injury induced by carbon tetrachloride in rats. This action was attributed to oleanolic acid content in kiwi fruit (**Liao *et al.*, 2007**). There are preliminary results of many studies support our result, as extract of kiwi fruit has the potential to boost the immune system by promoting bone marrow cell proliferation thus lead to increase immune function (**Lee *et al.*, 2010**). Previously, Kiwi fruit was reported to contain a number of anti-oxidant constituent and polyphenolic compounds, such as vitamin C, vitamin E, epicathechin, caffeic acid, quercetin and naringenin which have a powerful antioxidant effect that reversed the liver damage induced by CCl4 and improved liver function tests (**Fiorentino *et al.*, 2009**). Kiwi fruit was investigated against kidney stones in rats; it showed significant improvement in reducing the formation of kidney stones this is due to kiwifruit contains high amount of potassium and magnesium (**Szeto *et al.*, 2002**).

Table (5): Effect of kiwifruit treatment on liver and kidney parameters on rats intoxicated by rats H_2O_2.

Groups	Parameters					
	AST (Iu/l)	ALT (Iu/l)	ALP (Iu/l)	Uric acid mg/dl	Creatinine mg/dl	Urea mg/dl
G1 (–ve)	80.80± 2.11 a	65. 21± 1. 78 a	96. 26± 3.97 a	1.53± 0.78 e	1.16± 1.18 e	27.88 ± 4.72 e
G2(+ve)	193.57 ±5.58 d	142.86± 3. 27 d	164.39 ± 6.49 d	3.20± 0.52 a	5.62± 2.47 a	39.93 ± 3.20 a
G 3 (KWF juice 100 mg/ kg bwt/day)	116.86 ± 4.75 b	74.76± 3. 13 b	119.33 ± 3.65 b	1.79± 0.72 bc	3.87± 1.32 b	34.14 ± 3. 29 b
G 4 (KWF powder 10 g / kg diet/day)	103.08 ± 4.28 b	72. 03± 3.43 b	124.74 ± 5.649 b	1.82± 0.57 b	2.83± 1.25 c	31.17 ± 3.59 c
G 5 (Mixture of oral 100mg / kg bwt/day & powder 10g /kg diet/day)	118.08 ± 4.28 b	65. 83± 3.43 b	114.74 ± 5.649 b	1.72± 0.93 c	2.72± 1.15 c	30.21 ± 3.43 d

Each value represent the mean of three replicates ±SD. Values in each column having different superscript are significant different at $p \leq 0.05$.

6- Effects of kiwi fruit on total antioxidants, malondialdehyde (MDA), superoxide dismutase (SOD and glutathione (GSH) levels in serum of rats.

As shown in Table 6, H_2O_2 administration caused an elevation of MDA levels, and a demotion in total antioxidants, SOD and GSH levels compared with negative control group (-ve). However the antioxidants biochemical parameters total antioxidant capacity and SOD showed a significant increase in the received kiwi fruit groups versus the corresponding positive group (+ve). Treatment with kiwi fruit with different forms markedly reversed the alterations in biochemical parameters induced by H_2O_2. Group four which was treated by kiwi fruit powder 10 g / kg/ diet/day had the best result in lowering the elevation of MDA level caused by administration of H_2O_2. Otherwise, the best results in reversing the toxicity caused by H_2O_2 was recorded for group five that treated by kiwi fruit mixture oral 100mg /

kg/bwt/rats/day. The most commonly indicator of lipid peroxidation is MDA which increases after exposing to oxidative stress. Antioxidant activity of kiwi fruit is referred to its polyphenolic compounds (**Latocha *et al.*, 2010**) which have been found as a great antioxidant agents to prevent various lipid-peroxidation.

A significant decrease in MDA level besides an increasing in SOD and total antioxidant capacity were remarked to rats received kiwi fruit in the present study. The results at the same trend with finding of (**Duttaroy and Jørgensen 2004**) reported that the level of MDA returned to normalcy bias in rats treated with kiwi fruit extract. Kiwi fruit shows the capability of preventing cell damage resulted from the unstable free radicals during normal metabolism also in scavenging free radicals linked with cardiovascular diseases, the detailed mechanism is yet to be understood but may be it contains phytochemical compounds playing a great role as antioxidants such as chlorophylls, zeaxanthin, lutein, flavonones and vitamin C (**Leontowicz *et al.*, 2016**). Kiwi fruit extracts containing a wide variety of polyphenols, which could be protective for human DNA against oxidative stress due to its antioxidant effect reported by other studies (**Sumera *et al.*, 2015**).

Chemical composition of kiwi fruit contains flavonoids and isoflavones which act as anti-carcinogenic, also have a neuro-protective function (**Dehghani *et al.*, 2006**). As those phytochemical compounds increased in extracts thus leads to increase in reducing oxidative stress power by radical-scavenging (**Bekhradnia *et al.*, 2011**).

Kiwi fruit extracts contain phenolic compounds which found to attributed in donation electrons to H_2O_2 which neutralize it to water molecule and eliminate it from body (**Scalzo et al., 2005**).

Table (6): Effect of kiwifruit treatment on biochemical analysis parameters on rats intoxicated by rats H_2O_2.

Groups	Parameters			
	Total antioxidants U/mg	**SOD U/mg**	**Malondialdehydnmol/ L**	**GSH mg/mg protein**
G1 (–ve)	3.38± 0.75 a	1.67± 0.1 a	6.47± 0.76 d	0.14± 0.01 a
G2(+ve)	1.17± 0.55 d	0.33± 0.01 d	18.35± 1.16 a	0.10± 0.01 d
G 3 (KWF juice 100mg/ kg bwt/ day)	2.98± 0.65 b	1.0± 0.04 b	12.21± 0.96 c	0.15± 0.04ab
G 4 (KWF powder 10 g / kg diet/day)	2.58± 0.80 b	1.2± 0.06 b	11.37± 0.52 b	0.13± 0.06ab
G 5 (Mixture of oral 100mg/ kg bwt /day & powder 10g /kg diet/day)	3.19± 1.80 a	1.33± 0.08ab	13.21± 0.96 c	0.18± 0.08ab

Each value represent the mean of three replicates ±SD. Values in each column having different superscript are significant different at $p \leq 0.05$.

CONCLUSIONS:

This study showed that kiwi fruit with different forms (powder and juice) could ameliorate the toxicity induced by H_2O_2, may be by enhancing the total antioxidant capacity and restoration of SOD activity and declining of MAD levels. Therefore, kiwi fruit could be a potential bioactive useful as a powerful antioxidant and immune boosting fruit. Further studies are recommended to explain the mechanism of kiwi fruit as a natural immune boosting.

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التعزيز المناعى والنشاط المضاد للاكسدة لفاكهة الكيوي على السمية المستحدثة ببيروكسد الهيدروجين في الفئران

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الملخص العربي

تعرف فاكهة الكيوي باسم عنب الثعلب الصيني وتعتبر مكمل غذائي طبيعي مع العديد من الفوائد الصحية. تهدف هذه الدراسة إلى تقييم تأثير تعزيز المناعة والتأثير المضاد للأكسدة لفاكهة الكيوي بثلاثة مستويات مختلفة للحد من السمية بواسطة بيروكسيد الهيدروجين (H_2O_2) في الفئران. وقد استخدمت فى هذه الدراسة خمسة وعشرون فأراً ، قسمت إلى خمس مجموعات (5 فئران لكل مجموعة) ؛ المجموعة الكنترول السالبة (v –)، والمجموعة الكنترول الموجبة (v +) المصابة ببيروكسيد الهيدروجين (100 مجم H_2O_2 / كجم / وزن الجسم / فأر / يوم) ، والمجموعات المعالجة بكلا من : عصير الكيوي (100 مجم / كجم / وزن الجسم / جرذان / يوم) عن طريق الفم ، و مسحوق الكيوي (10) جم / كجم / غذاء / يوم) ،و خليط من كل من العصير ومسحوق الكيوي بنسب (100 مجم / كجم / وزن الجسم / فأر / يوم)(10 جم / كجم / دايت / يوم). في نهاية التجربة ، تم التضحية بجميع الفئران وتم تحليل مصلها. وأظهرت النتائج أن جميع المجموعات المعالجة بفاكهة الكيوي عصير ومسحوق يقلل بشكل كبير من سمية بيروكسيد الهيدروجين عن طريق تقليل نسب كلا (MDA، AST، ALT ، الكرياتينين ، اليوريا وحمض البوليك) وزيادة مستويات GSH، SOD ، مضادات الأكسدة الكلية ، بارامترات الدم والمعلمات الغذائية في مصل جميع المجموعات التي تلقت فاكهة الكيوي مقارنة مع بالمجموعة الضابطة الموجبة . توصلت الدراسة الى ان تحتوي فاكهة الكيوي عصير ومسحوق بمستويات مختلفة على بعض التأثيرات المعززة للمناعة والمضادات الأكسدة.

الكلمات المفتاحية: فاكهة الكيوي و المناعة و مضادات الاكسدة و مركبات كيميائية نباتية و مضادة للالتهابات.