

Combined Effects of Methionine and Kiwi Fruit on Paracetamol Induced Liver Injury

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Abstract: In the present study, the effects of methionine and kiwi fruit powder on paracetamol induced liver damage were evaluated. Forty adult male of white albino rats (Wistar rats strain) were randomly classified into five groups (8 rats each). The first group kept as control negative. The rest rats were administered paracetamol to induce liver injury and classified into control positive and treated groups which were methionine, kiwi powder and kiwi powder with methionine groups. Liver aspartate and alanine aminotransferase (AST, ALT) activity, alkaline phosphatase (ALP) and gamma glutamyl transferase (γ GT) enzymes as well as total bilirubin, total protein, albumin and globulin were determined in serum. Liver lipids parameters (cholesterol, total lipids, triglyceride and glycogen), Liver antioxidant enzymes (superoxide dismutase, SOD, glutathione peroxidase, GPX, glutathione S-transferase, GST and catalase) were estimated. Paracetamol administration showed a significant decrease in nutritional results; liver antioxidant enzymes; liver triglyceride and liver glycogen but showed a significant increase in liver and renal function parameters enzymes and MDA as shown in control positive group. Liver injured rats groups which fed diet contains methionine or kiwi powder or methionine with kiwi powder had a significant increase in body weight gain, food intake and feed efficiency ratio (FER) compared to rats of control positive group. Consumption of methionine with kiwi powder showed a significant improves of liver function enzymes, liver antioxidant enzymes and liver lipid pattern compared to rats of control positive group that is may be due to its augmenting endogenous antioxidant mechanisms.

Key words: Kiwi fruit • Methionine • Paracetamol • Rats

INTRODUCTION

Liver is the key organ of metabolism, excretion and detoxification of environmental toxicants. Liver damage is associated with distortion of several metabolic functions. Numerous medicinal plants and their formulations are used for liver disorders [1]. Acetaminophen (Paracetamol) is a widely used antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses. An oxidation product of acetaminophen is N-acetyl p-benzoquinamine which binds sulphydryl groups of proteins resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity. Hepatic cells appear to participate in a variety of enzymatic metabolic activities and acetaminophen produced marked liver damage [2]. Epidemiological studies have indicated possible

relationships between fruits and vegetable rich diet and reducing the risk of chronic diseases, especially coronary heart disease, diabetes mellitus and some malignancies [3]. The kiwifruit (*Actinidia deliciosa*) is a small fruit approximately 3 inches long and has a brown hairy peel with a green flesh and white pulp in the center with many tiny black edible seeds. Its green flesh is almost creamy in consistency with an invigorating taste reminiscent of strawberries, melons and bananas, yet with its own unique sweet flavor. Kiwifruit contains numerous phytonutrients as well as well known vitamins and minerals that promote health [4]. Kiwi fruit has incredible health benefits. Studies revealed that diseases like asthma cough and diabetes have shown positive improvements with the daily consumption of kiwi fruit. The great amount of dietary fiber in kiwi fruit helps in decreasing the probability of colon cancer. Kiwi's antioxidant properties help in protecting the body against free

radicals. The flavonoids present in kiwi fruits protect the cells from oxidative damage and in turn, help in guarding the DNA from mutation and damage [5, 6]. Methionine is an amino acid commonly found in animal-based protein and some fruits and vegetables and is considered an essential nutrient because body doesn't synthesize it. Methionine was needed for protein, especially muscle fibers, connective tissue and enzymes that needs for healthy metabolism and growth [7]. Methionine is also lipotropic compound that helps with metabolism and breaks down fat. It can also help with chelation, which is the removal of heavy metals from the body to ensure that the liver, kidneys and bladder remain healthy. This amino acid preserves artery function and maintains healthy nails, hair and skin [8].

The present study aimed to investigate the effect of consumption of kiwi fruit and methionine on paracetamol induced liver toxicity in experimental rats.

MATERIALS AND METHODS

Materials:

- Kiwi fruit was obtained from local market, washed with tap water and dried at 60°C then crushed to a fine powder and added to basal diet as 10%.
- Paracetamol drug was obtained from Kahira Pharm & Chem. Ind. Co. Cairo- Egypt. The rat received a single dose of 2 g/kg by stomach tube to induce liver injury [9].
- Methionine powder was purchased from El Gomhoria Company. Methionine powder was added to basal diet as 5 g/kg.
- Forty adult male of white albino rats (Sprague dawley strain), weighing 160± 5g, provided from of National Research Center, Dokki, Giza, Egypt. Rats were housed as groups in wire cages under the normal laboratory conditions.
- The basal diet: The rat basal diet was performed according to Reeves *et al.* [10].
- Kits for biochemical analysis were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods:

Biological Design: Food and water were provided *ad-libitum* to experimental animals for a week as adaptation period. The rats were randomly classified into five groups (8 rats each). The first group kept as control

negative. The rest of rats were administered paracetamol to induce liver injury and classified into control positive and treated groups which were methionine, kiwi powder and kiwi powder with methionine groups. The experiment continued for 60 days. Daily food intake (FI) and weekly body weight gain (BWG) were recorded. Feed efficiency ratio (FER) and protein efficiency ratio (PER) were determined by Chapman *et al.* [11]. At the end of experiment, rats were anesthetized and blood sample were collected from hepatic portal vein in clean centrifuge tubes. Liver was removed and blotted on filter paper.

Biochemical Analysis: The blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum aspartate and alanine aminotransferase (AST, ALT) activity, alkaline phosphatase (ALP) and gamma glutamyl transferase (γ GT) enzymes activity were estimated according to Reitman and Frankel [12], Draper and Hadley [13] and Kind and King [14], respectively. Serum total bilirubin, total protein, albumin and globulin were determined according to Jendrassik [15], Weichselbaum [16], Bartholomev and Delany [17] and Coles [18], respectively. Liver cholesterol, total lipids, triglyceride and glycogen were determined according to Richmond [19], Folch *et al.* [20], Scheletter and Nussel [21] and Rerup and Lundquist [22], respectively. Liver superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST), catalase and malondialdehyde (MDA) were estimated according to Beuchamp and Fridovich [23], Weiss *et al.* [24], Ellman [25], Cohen *et al.* [26] and Uchiyama and Mihara [27], respectively.

Statistical Analysis: Data are expressed as mean \pm SD. Statistical analysis was done by using analysis of variance (ANOVA) followed by student's t-test and P values of 5% and less were considered to be significant according to Artimage and Berry [28].

RESULTS

The results of body weight gain, food intake, feed efficiency ratio and protein efficiency ratio of the experimental rats were presented in Table 1. Obtained data showed that the control positive group had highly significant decrease in body weight gain, FI, FER and PER at $p < 0.001$ compared to rats of control negative group. Liver injured rats groups which fed diet contains methionine or kiwi powder or methionine with kiwi powder

had significant decrease in BWG ($p<0.001\&0.01$), FER ($p<0.001\&0.01$) and PER ($p<0.01$) compared to rats of control negative group but had significant increase in BWG, FI, FER and PER compared to rats of control positive group. The treatment effect of methionine or kiwi powder or methionine with kiwi powder consumption on serum ALT, AST, ALP and γ GT was represented in Table 2. The activities of serum ALT, AST, ALP and γ GT enzymes were highly significant increased at $p<0.001$ in control positive group. Consumption of methionine or kiwi powder could increase ALT, AST, ALP and γ GT at $p<0.01$ compared to rats of control negative group but could decrease in these parameters compared to rats of control positive group. However, Consumption of methionine with kiwi powder could improve liver function in paracetamol induced liver injury and appeared within normal.

The activities of serum total bilirubin and A/G ratio were significantly increased at $p<0.001\&0.01$, respectively and significant decrease in total protein and globulin at $p<0.01\&0.001$, respectively in control positive group compared with control negative group. Consumption of

methionine could lower total bilirubin compared to control positive group but the values of protein, albumin, globulin and A/G were within normal. Kiwi powder consumption could lower total bilirubin and increase the globulin value compared to control positive group but values of protein, albumin and A/G were within normal. However, Consumption of methionine with kiwi powder showed a non significant difference in these parameters as shown in Table 3.

Paracetamol administration could increase the levels of liver cholesterol and total lipids and decrease triglyceride and glycogen at $p<0.001$. However, consumption of methionine or methionine with kiwi powder showed normal values of these parameters. Consumption of kiwi powder showed a significant increase in glycogen compared to control positive as illustrated in Table 4. Paracetamol administration showed lower values of antioxidant enzymes as liver SOD, GPX, GST and catalase but showed higher value of MDA at $p<0.001$ as shown in control positive compared to control negative group. Consumption of methionine or methionine with kiwi powder showed a significant

Table 1: Mean values \pm SD of BWG (g), FI (g), FER and PER of the experimental rat groups.

Groups					
Variables	Control negative	Control positive	Methionine	Kiwi powder	Methionine +Kiwi powder
BWG	95.71 \pm 8.41 ^a	41.88 \pm 5.14 ^{d***}	61.43 \pm 6.13 ^{c***}	66.65 \pm 5.59 ^{bc**}	74.21 \pm 8.22 ^{b**}
FI (g)	16.85 \pm 1.84 ^a	11.73 \pm 1.17 ^{c***}	14.99 \pm 1.45 ^{ab}	15.21 \pm 1.22 ^a	16.81 \pm 1.71 ^a
FER	0.094 \pm 0.001 ^a	0.059 \pm 0.003 ^{d***}	0.068 \pm 0.005 ^{bc***}	0.073 \pm 0.004 ^{b**}	0.073 \pm 0.002 ^{b**}
PER	0.473 \pm 0.05 ^a	0.298 \pm 0.02 ^{c***}	0.342 \pm 0.03 ^{b**}	0.365 \pm 0.04 ^{b**}	0.368 \pm 0.01 ^{b**}

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$.

^{abcd} Mean values in each raw having similar letters were not significantly different

Table 2: Mean values \pm SD of serum ALT, AST, ALP and γ GT (μ l/ml) of the experimental rat groups.

Groups					
Variables	Control negative	Control positive	Methionine	Kiwi powder	Methionine +Kiwi powder
ALT	61.15 \pm 7.25 ^{de}	128.75 \pm 15.16 ^{a***}	90.77 \pm 9.31 ^{bc**}	101.21 \pm 12.41 ^{b**}	75.88 \pm 8.24 ^d
AST	52.37 \pm 6.14 ^d	99.11 \pm 9.22 ^{a***}	72.14 \pm 7.76 ^{b**}	79.14 \pm 8.41 ^{b**}	65.54 \pm 7.13 ^{cd}
ALP	70.36 \pm 8.45 ^c	137.35 \pm 13.77 ^{a***}	95.14 \pm 10.11 ^{b**}	105.21 \pm 11.41 ^{b**}	78.79 \pm 9.61 ^c
γ GT	7.34 \pm 1.21 ^c	12.69 \pm 1.73 ^{a***}	9.11 \pm 1.32 ^{b**}	9.35 \pm 1.61 ^{b**}	7.11 \pm 1.10 ^c

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$.

^{abcd} Mean values in each raw having similar letters were not significantly different.

Table 3: Mean values \pm SD of serum bilirubin, protein, albumin, globulin (g/dl) and A/G of the experimental rat groups.

Groups					
Variables	Control negative	Control positive	Methionine	Kiwi powder	Methionine + Kiwi powder
Bilirubin	1.39 \pm 0.19 ^c	2.59 \pm 0.45 ^{a***}	1.91 \pm 0.31 ^{b**}	1.78 \pm 0.38 ^{b**}	1.24 \pm 0.20 ^c
Protein	7.84 \pm 1.54 ^a	5.44 \pm 1.36 ^{b**}	6.36 \pm 1.32 ^a	6.01 \pm 1.45 ^a	7.38 \pm 1.60 ^a
Albumin	3.74 \pm 0.77 ^a	3.54 \pm 0.53 ^a	3.01 \pm 0.35 ^{ab}	3.11 \pm 0.40 ^{ab}	3.59 \pm 0.66 ^a
Globulin	4.12 \pm 1.01 ^a	1.95 \pm 0.16 ^{c***}	3.35 \pm 0.61 ^{ab}	2.94 \pm 0.33 ^{b**}	3.79 \pm 0.78 ^a
A/G	0.90 \pm 0.03 ^{bc}	1.81 \pm 0.29 ^{a**}	0.89 \pm 0.01 ^c	1.05 \pm 0.11 ^b	0.94 \pm 0.02 ^{bc}

Significant with control group * $P<0.05$ ** $P<0.01$ *** $P<0.001$.

^{abcd} Mean values in each raw having similar letters were not significantly different.

Table 4: Mean values \pm SD of liver cholesterol, total lipid, triglyceride and glycogen (mg/g) of the experimental rat groups.

Variables	Groups				
	Control negative	Control positive	Methionine	Kiwi powder	Methionine + Kiwi powder
Cholesterol	5.30 \pm 1.11 ^{bc}	9.35 \pm 1.01 ^{a***}	6.96 \pm 1.39 ^b	6.19 \pm 1.03 ^b	6.11 \pm 1.36 ^b
Total lipid	57.14 \pm 6.11 ^b	73.43 \pm 5.41 ^{a***}	61.61 \pm 5.12 ^b	62.18 \pm 7.21 ^b	60.17 \pm 7.21 ^b
Triglyceride	2.77 \pm 0.76 ^a	1.52 \pm 0.22 ^{c***}	2.11 \pm 0.64 ^b	2.07 \pm 0.38 ^b	2.63 \pm 0.75 ^b
Glycogen	4.1 \pm 0.77 ^a	2.99 \pm 0.33 ^{c***}	3.89 \pm 0.65 ^{ab}	3.45 \pm 0.55 ^{b*}	4.36 \pm 0.78 ^a

Significant with control group * P<0.05 ** P<0.01 *** P<0.001

^{abcd} Mean values in each raw having similar letters were not significantly different.Table 5: Mean values \pm SD of liver SOD, GPX, GST, catalase and MDA of the experimental groups.

Variables	Groups				
	Control negative	Control positive	Methionine	Kiwi powder	Methionine+Kiwi powder
SOD(mmol/l)	71.69 \pm .55 ^a	32.59 \pm 3.51 ^{b***}	70.11 \pm 7.21 ^a	74.33 \pm 7.19 ^a	75.14 \pm 6.11 ^a
GPX(mmol/l)	58.30 \pm 6.11 ^a	28.11 \pm 3.49 ^{c***}	51.21 \pm 5.96 ^a	49.38 \pm 5.71 ^{ab}	55.11 \pm 6.22 ^a
GST(mmol/l)	1.11 \pm 0.34 ^a	0.44 \pm 0.03 ^{d***}	0.85 \pm 0.04 ^{c**}	0.80 \pm 0.04 ^{c**}	0.98 \pm 0.03 ^{b**}
Catalase(μ /l)	43.14 \pm 4.21 ^a	19.41 \pm 1.73 ^{d***}	35.11 \pm 3.41 ^{ab}	31.21 \pm 3.11 ^{bc**}	41.22 \pm 5.14 ^a
MDA(mmol/l)	9.67 \pm 1.50 ^d	23.77 \pm 4.11 ^{a***}	14.61 \pm 2.11 ^{b**}	15.96 \pm 1.51 ^{b**}	11.32 \pm 1.36 ^{c*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001.

^{abcd} Mean values in each raw having similar letters were not significantly different.

decrease in GST and MDA at $p<0.01$ & 0.05 but consumption of kiwi powder showed a significant decrease in GST, catalase and MDA at $p<0.01$ compared to control negative group. Consumption of methionine or kiwi powder or methionine with kiwi powder showed a significant increase in liver SOD, GPX, GST and catalase and a significant decrease in liver MDA compared to control positive group.

DISCUSSION

The obtained nutritional results were revealed to the fact that the covalent binding of an oxidation product of paracetamol is N-acetyl-p-benzoquinoneimine and sulphhydryl groups of protein results in cell necrosis and lipid peroxidation which causes hepatotoxicity [2, 29]. Researchers have found that methionine is also one of the three amino acids needed by the body to manufacture creatine monohydrate, a compound essential for energy production and muscle building. Low levels of methionine can slow normal growth and development [30]. Kiwi fruit is one of the most nutritious fruits. Kiwi fruit is also rich in nutrition. The fruit contains vitamin C, vitamin E, vitamin A, beta carotene and potassium. Vitamin C can strengthen the immune system, accelerate the healing of wound and promote the absorption of iron. Tiny black edible seeds of kiwi fruit produce fruit oil which is rich in alpha-linoleic acid (an important omega-3 essential fatty acid). The fruit skin is a rich in flavonoid antioxidants [31, 32].

It has been well established that elevated levels of ALT, AST and ALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. Serum total protein and bilirubin levels on the other hand are related to the function of the hepatic cells revealing the functional status of the hepatic cell [33, 34]. Bilirubin is one of the most clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. In the present study paracetamol hepatotoxic rats showed a significant increase in the level of serum total bilirubin when compared with control rats [35]. Methionine is a great antioxidant as the sulfur it supplies inactivates free radicals. It may also be used to treat depression, arthritis pain as well as chronic liver disease - although these claims are still under investigation. The most common medical use of this amino acid is as a preventative treatment for liver damage caused by acetaminophen poisoning [8]. Kiwi contains a large number of arginine, which can promote the blood circulation and enhance the working efficiency. Kiwi fruit almost contains no fat, but it contains plenty of pectin and vitamin E, which are very beneficial for the health of heart. At the same time, it can also lower the level of cholesterol [36]. Kiwi fruit contains great amounts of healthy dietary fiber and a variety of antioxidants. As a result, it can effectively prevent and treat liver disease [37]. Kiwi can prevent the increase of plasma cholesterol, LDLc, triglyceride levels and, of increasing HDLc levels in rats fed with high fat diet.

The decrease of serum MDA levels and the increase of activity of SOD were significant in rats adopting kiwi compound oral liquid or exercise training before the hyperlipidemia model was established while the data were not marked in hyperlipidemic rats. Studies have shown that kiwi is beneficial for reducing AMD. This effect of kiwi fruits is attributed to the flavonoid antioxidants like beta carotene, lutein and xanthin [38].

Administration of paracetamol resulted in significant increase in serum and hepatic SOD and glutathione peroxidase (GPx) activities with a significant decrease in blood and hepatic glutathione (GSH) levels [39]. The enzymatic antioxidant defense system is the nature protector against lipid peroxidation. SOD, catalase and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage. The observation that increasing antioxidant enzymes after consumption of diet containing methionine and kiwi was identical with that of Selvam and Kurien [30], who reported that feeding methionine, reduced the susceptibility for lipid peroxidation by restoration of the level of free radical scavengers. Seneviratne *et al.* [7] reported that methionine has a significant effect on the myocardial antioxidant enzyme activities and only changes in glutathione peroxidase enzyme activity correlated with the mRNA changes. These antioxidant changes may have a role in the beneficial effects of methionine in pathological rather than physiological conditions. Methionine benefits liver health and its natural detoxification functions. This sulfur-containing amino acid helps aids the liver in processing fats, which prevents fatty liver disease. Also by increasing glutathione levels, methionine helps the liver to effectively neutralize toxins. Methionine naturally supports cellular health with its powerful antioxidant activities.

A chemical analysis identified ascorbic acid and numerous other potential antioxidants in kiwi fruit. These included R-tocomenol, a vitamin E analog, tocopherol, R-tocopherol, caffeic acid glucosyl derivatives, Alpha-sitosterol, chlorogenic acid and flavones and flavonones. The antioxidant capacity of kiwifruit constituents has monitor the quenching, scavenging, or retarding of free-radical generation [40]. Kiwifruit juice was observed to be a potent inhibitor of lipid oxidation and an effective eliminator of the oxidative stress inducing agent hydrogen peroxide (H₂O₂). The juice also possessed superoxide dismutase like activity and acted as a copper-reducing agent *in vitro* [41].

CONCLUSION

In conclusion, the results of this study demonstrate that, both kiwi and methionine exhibit a potent action against paracetamol induced hepatic damage in rats. Such effects can be correlated directly with its ability to reduce lipid peroxidation and enhance the antioxidant defense status.

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