



Biochemical Effect of Fisetin on Experimentally Induced Liver Damage in Rats

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ABSTRACT

Fisetin (3, 7, 3', 4'-TetrahydroxyFlavone) is a bioflavonoid found in fruits and vegetables. It displays a wide variety of pharmacological properties, including antioxidant, anti-carcinogenic and anti-inflammatory effects. This study was done to explore the role of fisetin, in ameliorating oxidative damage in thioacetamide (TAA)-induced hepatic injury in rats. Thirty-six male albino rats were divided into three equal groups. Group I (normal control group): rats administered distilled water only. Group II (TAA-intoxicated group): rats received thioacetamide (50 mg/kg b. wt.) intraperitoneally twice weekly for 6 weeks. Group III (TAA + fisetin co-treated group): rats received thioacetamide (50 mg/kg b. wt.) and at the same time administered fisetin (10mg/ kg b. wt. /daily /orally) for 6 weeks (end of experiment). All animals were sacrificed after 6 weeks. The results revealed that serum levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) were significantly elevated in Group II. Oxidative stress in the group II was manifested by a significant rise in Malondialdehyde (L-MDA) levels with a marked reduction in Glutathione (GSH) content and diminished activity of antioxidant enzyme Glutathione-S-Transferase (GST), in liver tissues as compared with the control group. The coadministration of fisetin and thioacetamide (protection modality) restored the thioacetamide induced alterations in liver functions, promoted oxidative stress and antioxidant defense. Thus, the results of the present study indicate that fisetin treatment protects the hepatocytes by improving the antioxidant competence in hepatic tissues of thioacetamide intoxicated rats.

Keywords: Thioacetamide, fisetin, oxidative stress, rats.

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1. INTRODUCTION

Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver injury (Li et al., 2015). Significant

advances in our understanding of mechanisms responsible for hepatotoxic injury have arisen from molecular, cellular and functional studies in animals

(Yogalakshmi et al., 2010). hepatotoxins initially damage the centrilobular regions of liver where there are high levels of cytochrome P450 mixed function oxidases that mediate their conversion to toxic intermediates, followed by reactive oxygen species (ROS) production, lipid peroxidation and release of pro-inflammatory cytokines (Luster et al., 2001).

Among the various toxicity models, thioacetamide (TAA) is frequently used to produce liver injury in animals and further to evaluate the therapeutic potential of drugs (Aydin et al., 2010). One of the mechanisms of damage is the generation of ROS and instigation of oxidative stress (Wang et al., 2000). After administration TAA undergoes extensive metabolism in the liver to acetamide and then to thioacetamide-S-oxide by the mixed function oxidase system. Thioacetamide-S-oxide is further metabolized, by cytochrome P- 450 monooxygenase to thioacetamide-S-dioxide that exerts hepatotoxicity by binding to macromolecules causing centrilobular necrosis and generation of ROS (Chilakapati et al., 2005). This eventually leads to oxidative stress by enhancing free radical-mediated damage to proteins, lipids and DNA (Li et al., 2015). Application of antioxidants are expected to signify a rational curative strategy to prevent and cure liver diseases involving oxidative stress. Although conclusions drawn from clinical studies remain uncertain, animal studies have revealed the promising in vivo therapeutic effect of antioxidants on liver diseases.

Natural antioxidants found in edible or medicinal plants often contains strong antioxidants and free radical scavengers as well as anti-inflammatory mediators, which are also thought to be the source of other bioactivities and health benefits and ability to

interact with many basic cellular activities (Finley et al., 2011, Bast and Grmm, 2015)

Due to their abundance in dietary products and their potential pharmacological and nutritional effects, the flavonoids are of considerable interest for drug as well as health food supplements (Khan et al., 2013)

Fisetin (3, 3', 4', 7-tetrahydroxyflavone), a naturally occurring flavonoid, is present in discernible quantities in many vegetables and fruits including strawberry, apple, persimmon, cucumber and onion. Fisetin was found to possess antioxidant, anti-inflammatory, anticancer, antihyperlipidemic, and neuroprotective properties (Chen et al., 2014, Jo et al., 2014). A recent investigation also revealed that fisetin protects the liver by attenuating oxidative stress during streptozotocin-induced hyperglycaemia (Prasath et al., 2014).

Although the antioxidant and anti-inflammatory properties of fisetin are well established, the effect of fisetin against TAA-induced hepatotoxicity was never explored. Hence, in the present study, we explored the possible beneficial effects of fisetin administration against thioacetamide - induced liver injury in male rats.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Thirty-six adult white male albino rats of 6-8 weeks old and weighing 150 – 200 g were used in this study. They were obtained from the breeding unit of Egyptian Organization for Biological Products and Vaccines (Abbassia, Cairo). Rats were housed in separated in steel mesh cages and kept under conventional laboratory conditions throughout the period of experiment. The rats were fed a standard rat

pellet diet and fresh, clean drinking water was supplied ad-libitum. Animals were maintained under standard conditions of ventilation, temperature ($25\pm 2^{\circ}\text{C}$), humidity (60-70%) and light/dark condition (12/12h).

All rats were acclimatized for a period of 15 days prior to the beginning of study. The local committee approved the design of the experiments, and the protocol conforms the guidelines of the National Institute of Health (NIH) and National Research Center-Medical Research Ethics Committee for the use of animal subjects.

2.2. Chemicals and antioxidant:

All chemicals were of analytical grade and obtained from standard commercial suppliers. The antioxidant and chemicals used in the present study were:

- a. Thioacetamide (TAA) extra pure was purchased from (Merck, Darmstadt Germany). TAA was prepared freshly by dissolving in sterile distilled water. The resultant suspension was administered ip to animals twice a week at a doses of 50 mg/kg b.wt. (Anbarasu *et al.*, 2012).
- b. Fisetin was purchased from (Abcam Chemical Co., St. Louis MO, USA) it was dissolved in 10%DMSO freshly prepared and administered orally to rats at a dose level of (10 mg/kg b. wt.) once daily (Koneru *et al.*, 2016)
- c. Other chemicals used in this study were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt and Bio-diagnostic Co., Cairo. Egypt.

2.3. Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (12 rats each)

placed in individual cages and classified as follow:

Group I (normal control group): Rats received no drugs, served as control non-treated for all experimental groups.

Group II (Thioacetamide intoxicated group): Rats were weighted and received thioacetamide at a dose level of (50 mg/kg b. wt.) intraperitoneally twice weekly for 6 weeks.

Group III (Thioacetamide + fisetin co-treated group): Rats were administrated fisetin (10 mg/kg b. wt./ orally, daily) and simultaneously administered thioacetamide (50 mg/kg b. wt.) intraperitoneally twice weekly 2h after the respective assigned treatment for 6 weeks.

2.4. Sampling:

2.4.1. Blood samples:

Twenty-four hours fasting after the last dose of the drugs treatment administration, rats were anaesthetized under diethyl ether anesthesia. Blood samples were collected by ocular vein puncture in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minutes. The serum was taken by automatic pipettes and collected in dry sterile tubes, then kept in deep freeze at -20°C until use for assay of the liver biomarker. All sera were analyzed for determination of the following parameters: AST, ALT, ALP and total bilirubin.

2.4.2. Tissue samples:

The rats were then sacrificed by cervical dislocation and the livers were immediately harvested, washed several times in normal saline, blotted with, blotted between two damp filter papers, weighed and stored at -80°C for subsequent biochemical analyses.

2.4.2.1. Liver tissue for biochemical analysis

Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volumes of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of L-MDA conc.

0.2 g of liver tissues were 0.2 g of liver tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clean supernatant was removed and used for determination of GSH concentration.

Prior to dissection, liver tissues were perfused with a PBS (phosphate buffered saline) solution, (pH 7.4), containing 0.16mg/ml heparin. 0.2 g of liver tissue was homogenized into 5 ml cold buffer (100Mm potassium phosphate, Ph7.0, containing 2 Mm EDTA) per gram tissue. then the supernatant was removed and used for GST activity assay.

2.5. Biochemical analysis

Serum ALT, AST were determined according to the method described by Schumann et al., (2002) and serum ALP activity was determined enzymatically according to EL-Aaser and EL-Merzabani, (1975). Liver tissue L-MDA and GSH were determined according to the method described by Mesbah et al., (2004) and Patterson and Lazarow, (1955) respectively.

2.6. Statistical analysis:

The results were expressed as mean \pm SE using SPSS software program version 16 (SPSS© Inc., USA). The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparison among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p < 0.05$.

3. RESULTS

Daily access to food and water was observed to be same during the experimental period. No difference in body weight gain was observed among all groups at the end of the study (data not shown). No mortality was seen in animals during the study.

Serum ALT, AST and ALP activities were significantly elevated ($P \leq 0.05$) in rats received TAA alone when compared with the control group. Fisetin treatment to thioacetamide intoxicated male rats significantly prevented these changes, resulting in a remarkable protection regarding the same parameters when compared with thioacetamide exposed group. With the ability to restore ALT and AST levels to the normal level of control group (Table 1).

The data summarized in table (2) revealed that, thioacetamide intoxicated rats showed significant increase in liver tissue L-MDA, when compared to normal control group. Administration of fisetin induce noticeable change in TAA intoxicated male rats causing a significant decrease in elevated liver tissue L-MDA level when compared with TAA toxic group.

In the experimental group that received TAA alone, there was a significant reduction in hepatic GST activity and hepatic reduced glutathione content GSH ($P \leq 0.05$) compared with control rats.

However, in the group that received TAA and fisetin, these hepatic antioxidant parameters were significantly elevated in comparison with the TAA group, returning to levels almost near to controls (Table 2).

Table (1): Effect of fisetin administration on serum ALT, AST and ALP activities in thioacetamide intoxicated male rats.

Parameters	ALT (U/L)	AST(U/L)	ALP(U/L)
Exp. groups			
Group I: Normal control	46±6.08 ^c	187.66±10.78 ^b	429.33±25.42 ^c
Group II: Thioacetamide (TAA)group	128.33±4.04 ^a	450.66±16.65 ^a	1133.66±48.54 ^a
Group III: TAA + fisetin	63±9 ^b	203±10.58 ^b	506±32.90 ^b

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (2): Effect of fisetin administration on liver tissue MDA, GST activities and GSH concentration in thioacetamide intoxicated male rats.

Parameters	L-MDA (nmol/ g tissue)	GST μ/g tissue	GSH μ/g tissue
Exp. groups			
Group I: Normal control	0.773±.047 ^c	87.88±.690 ^a	7.74±.769 ^a
Group II: Thioacetamide (TAA)group	1.59±.153 ^a	63.97±5.84 ^b	4.40±.140 ^b
Group III: TAA + fisetin	1.28±.060 ^b	86.27±5.63 ^a	6.71±.700 ^a

Data are presented as (Mean ± S.E). S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

4. DISCUSSION:

Foods rich in bioactive phytochemicals derived primarily from fruits and vegetables not only promote the health of an individual but also make them a positive addition to the human diet (Hasler, 2002). Flavonoids, a group of bioactive polyphenolic phytochemicals, due to their abundance in plant derived diets and their potential beneficial pharmacological effects, play an imperative role in health food supplements (Hasler, 2002). Strawberries, persimmons, apples, grapes, kiwi fruits, cucumber and onions are some of the commonly consumed fruits throughout the world and fisetin represents one of the bioactive phytochemical in them (Koneru et al., 2016).

In the present study, we investigated the hepatoprotective effect of fisetin using thioacetamide -induced liver injury model in rats.

Serum biomarkers are the preliminary indicators of hepatic damage often before the appearance of other relevant liver injury symptoms. Elevated serum ALT levels indicate reversible hepatocyte damage and leakage of cellular components into the serum, whereas increase in the levels of AST indicates hepatic necrosis and mitochondrial damage (Al-Attar, 2011). ALP is excreted normally via bile by the liver. In the liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in the increased activity of serum ALP (Singh et al., 1998).

In the present study, administration of thioacetamide significantly elevated the serum transaminases, ALP and total bilirubin activities when compared to the normal rats. This result is in agreement with previous

data by Esmat et al. (2013) who reported that, TAA intoxication caused dramatic increases in ALT, AST and ALP activities and the AST/ALT ratio compared with normal control rats.

Meanwhile, there was a significant restoration of these enzyme levels on fisetin administration. These findings are supported by several other studies (Koneru et al., 2016). Serum levels of transaminases return to normal may be a consequence of healing of hepatic parenchyma, stabilization of plasma membrane regeneration of hepatocytes, as well as repair of hepatic tissue damage caused by TAA (Moustafa et al., 2014).

Lipid peroxides, hydroperoxides and protein carbonyls are the oxidative stress markers that are elevated as a result of the toxic effect of reactive oxygen species (Fazal et al., 2014). MDA has the capability to interact with and bind to proteins, potentially rendering a vital protein to be nonfunctional. MDA induce oxidative stress by targeting mitochondrial complexes I and II and thereby disrupting proper flow of electrons through the electron transport chain (Long et al., 2009). Significant elevated levels of MDA (indicator of lipid peroxidation) have been observed in rat liver exposed to TAA. In several studies, TAA administration resulted in a noticeable increase in liver MDA levels (Aydin et al., 2010, Alshawsh et al., 2011). The observed significant normalization in the levels of L-MDA might be due to the anti-lipid peroxidative nature of fisetin (Khan et al., 2013, Prasath et al., 2014).

The non-enzymatic anti-oxidant GSH is thought to be a vital player in the process of detoxification, which energetically participates in reactions which lead to the obliteration of H₂O₂, free radicals, and other foreign compounds (Kamanli et al., 2004). The heavy

depletion of GSH to scavenge the toxic intermediates formed by TAA led to considerably lower levels of GSH content in liver tissue of the TAA group. On the other hand, a significant rise in GSH level was observed in the case of fisetin administration to TAA-induced liver toxicity. This result agrees with recent literature content (Khan *et al.*, 2013, Prasath *et al.*, 2014, Koneru *et al.*, 2016)

The GST superfamily comprises enzymes that protect healthy cells from reactive oxygen species (ROS), exogenous toxins, and cytotoxic agents. Inactivation of GST can create a deficit of detoxification capacity and increase tissue susceptibility towards carcinogens and ROS (Maruyama *et al.*, 2002). The present study indicated a significant reduction in hepatic GST activity when compared with normal control group. These findings concur with that of Spira and Raw, (2000) who supposed that GST activity inhibition is due to the deleterious effect of TAA on GST transcription. Treatment of fisetin (10mg/kg) markedly restored the TAA induced decrease in hepatic GST activity at ($P \leq 0.05$). This result was supported by (Khan *et al.*, 2013, Sahu *et al.*, 2014, Syed *et al.*, 2016).

5. CONCLUSION

The results from the present study revealed that co-treatment with fisetin at 10 mg/kg can alleviate the deleterious effects of thioacetamide in liver tissues. Fisetin administration restored the TAA-induced liver functions alteration and antioxidants defenses through its antioxidant and hepatoprotective potential.

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