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Efficiency of Hesperidin Against Liver Fibrosis Induced by Thioacetamidein Rats

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Abstract.

Hesperidin,a food derived antioxidants hase effect as chemo preventive agents in disease states involving oxidative stress, such as hepatitis. This study was done to clarify the effect of Hesperidin (HES) in protecting the liver from the bad effect of Thioacetamide (TAA) . Forty-male Wister rats were divided randomly into four equal groups (10 rats in each) and treated for 42 days as follow:Group I (control -ve) received normal saline 1 ml/kg BW, group II (control +ve) received TAA 100 mg/kg B.W by i/p injection intraperitoneally (i/p), group III has received TAA 100 mg/kg B.W injection i/p plus HES 100 mg/kg B.W orally, and Group IV received TAA 100 mg/kg B.W injection i/p plus HES 200 mg/kg B.W orally. At the end of the experiment, all animals were weighted, sacrified for blood and liver samples collection. The results of this study showed significant decrease ($P<0.01$) in B.W. and the level of SOD and CAT of the TAA treated groups in addition to significant increase ($P>0.01$) in MAD levels. Although Hesperidin administration at doses 100 & 200mg/kg showed improvement on these measurements, however 200 mg/kg was the more effective. The histopathological change resembled by excessive fibrous connective proliferation in group II were improved by HES 100 , 200 mg/kg B.W. In conclusion, HES counteract the hepatic oxidative stress induced by TAA in a dose of 200 mg/kg B.W

INTRODUCTION:

Thioacetamide (TAA) is a fungicide widely used to prevent decay in fruits, although it is classified as a group 2A human carcinogen[1]. TAA is widely employed to induce fibrosis in animal models because it results in morphological and biochemical characteristics similar to those observed in human liver cirrhosis[2,3]. TAA produces damage not only in the liver, but also other organs[4], and is metabolized by hepatic cytochrome P450 (Cyt-P450) to generate TAA S-dioxide[5]. This active product induces oxidative stress and production of TGF-β and reactive oxygen species (ROS)[6]. TAA-induced liver fibrosis is established when hepatic stellate cells (HSCs) areactivated through lipid peroxide formation, cytotoxicity, and mitochondrial injury. Taken together, these events may lead to apoptosis, necrosis, formation of cholangiocarcinoma, and excessive accumulation of extracellular matrix proteins (ECM)[7].

METHOD:

Experimental design:

The present study has been conducted in the animal house of the college of veterinary medicine, AL-Qasim Green University during the period extended from 29 december 2019 to 10 February, 2020. Forty-male Wister rats were divided randomly into four equal groups (10 rats in each) and treated for 42 days as follow: Group I (control -ve) received normal saline 1 ml/kg BW, group II (control +ve) received TAA 100 mg/kg B.W by i/p injection intraperitoneally (i/p), group III has received TAA 100 mg/kg B.W injection i/p plus HES 100 mg/kg B.W orally, and Group IV received TAA 100 mg/kg B.W injection i/p plus HES 200 mg/kg B.W orally. At the end of the experiment, all animals were weighted, sacrified for blood and liver samples collection. Anti oxidant / oxidant status. Plasma concentration of MDA, SOD, and CAT were determined using commercial kit provided

Histopathological examination:

Serial histological sections of 4 to 5 μm thickness of formalin preservative liver tissue were subjected to hematoxylin and eosin (HE)[8,9]

Statistical analysis

The statistical analysis was carried by using Complete Randomized Design (CRD) method, according to[10,11].The mean differences between the averages of the studied traits were determined at the probability level of (0.01) using the Duncan test[12] Statistical data were analyzed using the [13](SAS, 2010).

RESULTS:

Effect of Hesperidin on Thioacetamide-induced change of the body weight:

In regard to body weight, results revealed that body weight increased significantly in ($P<0.01$) all TAA treated groups. Hespiridine improve body weight in ascending manner with dose level in TAA_HES100, and TAA+HES200.

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Table No: (4-1):Effect of Hesperidin on Thioacetamide-induced change of the body weight /gram of rats. Mean ± SE, no= ?

Group / Parameters	(Control)	(TAA)	(TAA+ HES100) mg	(TAA+ HES200) mg
No. of rats	10	10	10	10
Weight at 1 day (g/animal)NS	180.450 ± 0.58 A	180.410 ± 0.67 A	180.420 ± 0.65 A	180.400 ± 0.78 A
Weight at 42 day (g/animal)**	236.290 ± 0.88 A	194.700 ± 0.57 D	213.100 ± 0.61 C	222.710 ± 0.86 B

Capital letters denoted significant differences between groups (rows). (P<0.01)

Body weight is considered as a simple and sensitive indicator of adverse effects after exposure to a substance[14]. The reduction in body weight of TAA exposed rats was in agreement with result of[15,16,17]. The decrease in body weight may be due to reduced adipose tissue and proteins , additionally, could be due to the direct effect of TAA on the decrease food intake behavior of the rats. TAA might have increased the protein catabolism and hampered the utilization of food consumed during the intoxication period, thereby causing a decrease in body weight[18,19]. Reduction in body weight in TAA-treated rats might be in part due to gastrointestinal toxicity and concomitant loss of the animal appetite with subsequent reduction of food ingestion or due to excessive loss of water, salts and proteins as a result of renal injury with subsequent dehydration and weight loss [20]. Increase in body weight of rats, after administration of HES in treated groups, when compared with TAA group, this result was in agreement with result of[21,22]. Gain in body weight was observed among the treated groups, may be due to HES is a major flavonoid,exhibits several pharmacological actions such as antihyperlipidemic, cardioprotective, antihypertensive, antidiabeticactivities, which are mainly attributed to an antioxidant defense mechanism and suppression of pro-inflammatory cytokine production. The beneficial effects of HES, which mainly results from anti-inflammatory [23,24].

Effect of Hesperidin on Thioacetamide-induced change of oxidant / antioxidant enzymes:

Results denoted to the significant increase in level ofMDA of TAA treated groups in compare with control. Dose of 100,and 200 mg/kg of hesperidin reduced MDA in G3&4. Antioxidant enzymes SOD &CAT were decreased significantly by TAA treatment but they were elevated by adiminstration of HES dose of 100, 200 mg/kg, (Table 2).

Table No:(4-3):Effect of Hesperidin on Thioacetamide-induced change on antioxidant enzymes of rats

Group / Parameters	(Control) Mean ± SE	(TAA) Mean ± SE	(TAA+ HES100)mg Mean ±SE	(TAA+ HES200)mg Mean ± SE
MDA (µmol/ml)	1.68 ± 0.006 D	3.95 ± 0.004 A	2.65 ± 0.004 B	1.95 ± 0.007 C
SOD (U/ml)	1.79 ± 0.004 A	0.82 ± 0.005 D	1.05 ± 0.003 C	1.54 ± 0.003 B
CAT (U/ml)	0.62 ± 0.005 A	0.25 ± 0.005 D	0.36 ± 0.005 C	0.53 ± 0.005 B

This study revealed aantioxidant role of hesperidin was in agreement with result of [25-30] MDA is an indicator of lipid peroxidation. Generation of these toxic metabolites after TAA metabolism resulted in the denaturation of cellular biomolecules such as lipids, resulting in lipid peroxidation[31]. TAA exerts a hepatotoxic effect by the production of ROS, peroxides, and free radicals [32].TAA was involving multiple mechanisms. For instance, TAA induces hepatocyte damage via its metabolite, TAA TASO2, which covalently binds to macromolecules of hepatocytes causing, protein oxidation and lipid peroxidation of the cell membrane biomolecules[33-34]. SOD and

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CAT activitiesare indicators of oxidative stress. The toxicity of TAA results from its hepatic bioactivation by CYP2E1 and Flavinmonooxygenases[35] This metabolic activation leads to the formation of reactive metabolites and ROS, generated as intermediates. Hence, the biotransformation of TAA precedes oxidative damage-associated liver injury[36]. The decrease in MDA and increase in SOD and CAT compared of HES treated groups was in agreement with result of [37]. HES reduced indicators of oxidative stress, such as ROS and lipid peroxidation, in a dose-dependent manner[38]. The anti-inflammatory and anti-fibrotic effects of HES can be explained, by the antioxidant properties described by[39] , who assessed the capability of HES to prevent cell damage through the augmentation of the cellular antioxidant defense system. HES has excellent antioxidant properties that it promoted antioxidant enzymes and inhibited ROS-induced oxidative damage.

Histopathological Study:

Microscopically section of the liver from control showed there is no Pathological changes with central vein and irradiated cord hepatic cells and sinusoids (Fig.4-1.a). Liver sections of TAA rats showed excessive fibrous connective proliferation and inflammatory cells infiltration (Fig.4-1.b).liver section examination of TAA+HES200 showed excellent regeneration in hepatocyte with increase infiltration of kupffer cells(fig.4-1.e).

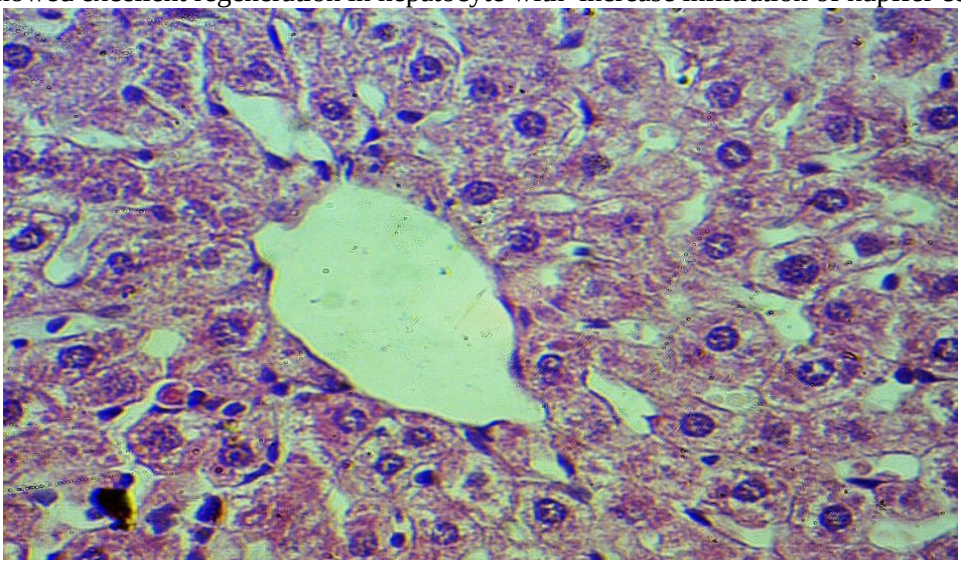

Fig. No: (4-1.a): Histopathological section of rat liver in G1 group showing the normal histological architecture (H&E.400X).

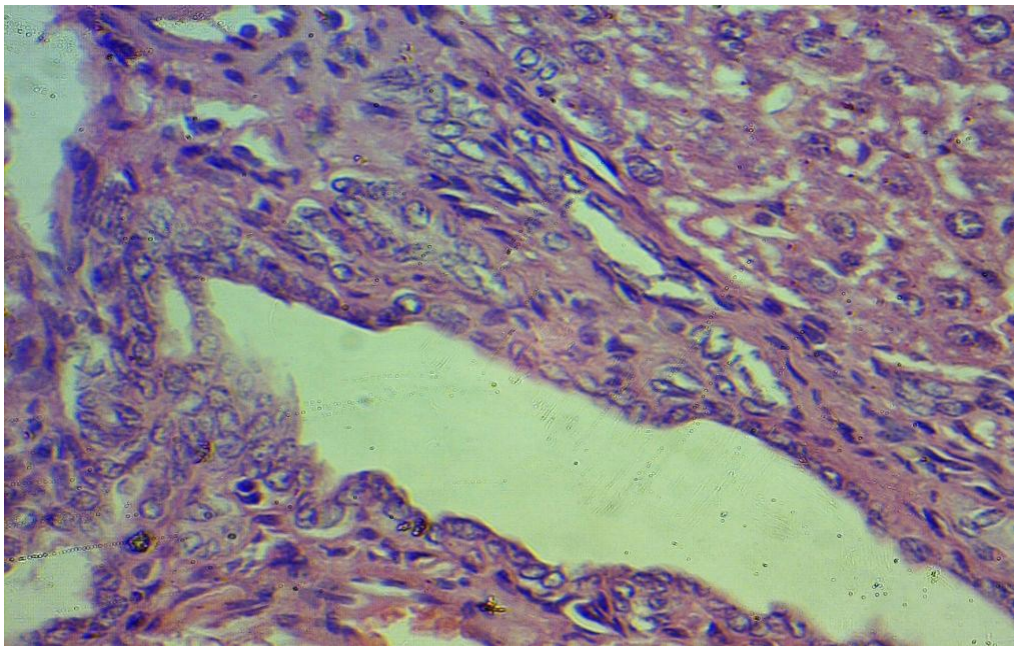

Fig. No: (4-1.b): Histopathological section of rat liver in G2 group which treated (i.p) with(100mg/kg B.w.)twice weekly thioacetamide for 6 weeks showing highly thickening of biliary canal due to excessive fibrous connective proliferation with hyperplasia of epithelial cells lining (H&E.400X).

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The result of TAA treated group showed there was excessive fibrous connective proliferation and inflammatory cells infiltration. The result was in agreement with result. Administration of TAA to rodents resulted in cell death by apoptosis and necrosis simultaneously. The mechanism behind this toxicity is associated with its toxic metabolites the s-oxide which it reduce the number of hepatocytes as well as rate of oxygen consumption and also decrease the volume of the excreted bile.Apoptosis induced by TAA is mediated through interensic or mitochondrial pathway as confirmed by several studies. TAA intoxication in experimental animals including cell necrosis and nodular cirrhosis.

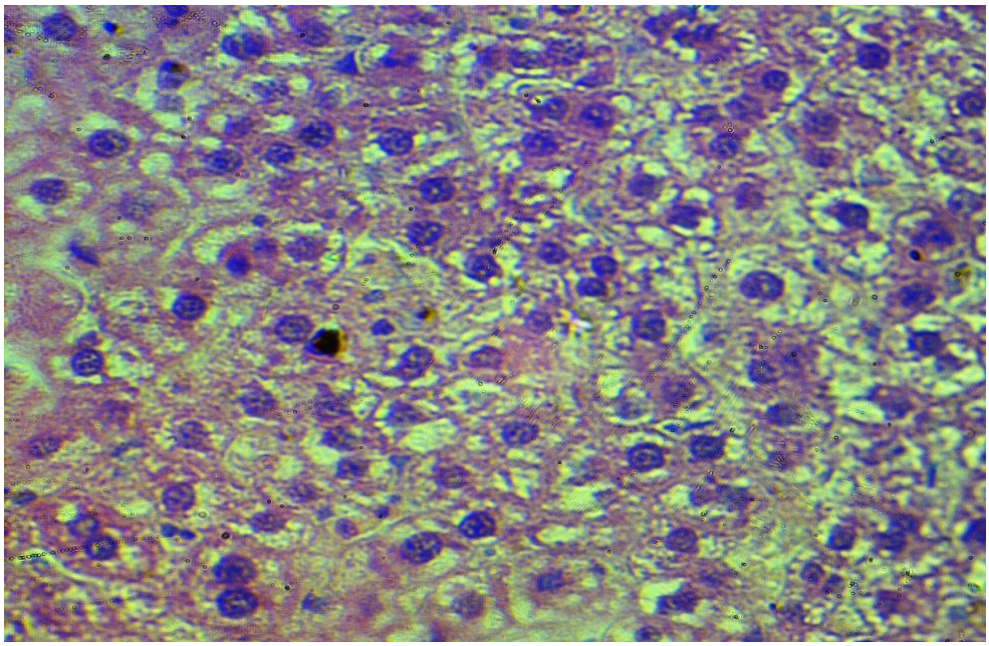

Fig. No: (4-1.c): Histopathological section of rat liver in G3 group which treated (i.p) with (100 mg/kg B.w.) twice weekly thioacetamide + HES(100 mg/kg B.w.) orally for 6 weeks showing mild regeneration in hepatocyte with some mitotic figure is also seen (H&400X).

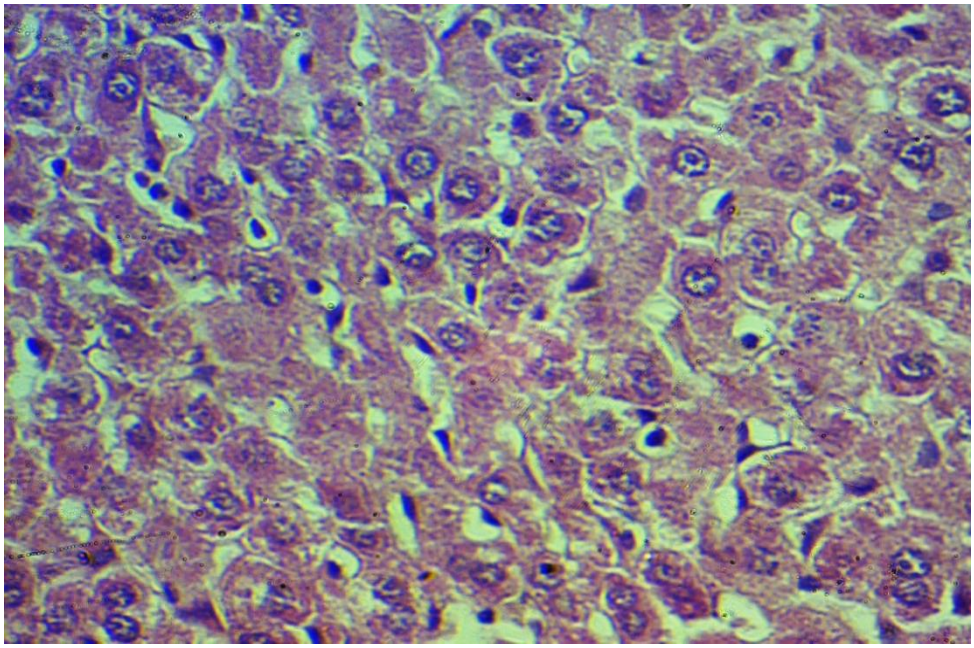

Fig. No: (4-1.d): Histopathological section of rat liver in G4 group which treated (i.p) with (100 mg/kg B.w.) twice weekly thioacetamide + HES(200 mg/kg B.w.) orally for 6 weeks showing excellent regeneration in hepatocyte with increase infiltration of kupffer cells (H&E.400X).

CONCLUSION

The result of G3 and G4 groups showed there was regeneration in hepatocyte with some mitotic figure is also

seen with increase infiltration of kupffer cells. The result indicating an improved histological appearance in the liver tissue. Administration of HES helped protect the hepatic tissues against the toxic effects of TAA and restored the levels of oxidative stress and antioxidant biomarkers in the liver to their normal state. The result in this study were in agreement with study. HES treatments, a significant decrease in hepatic hydroxyproline suggesting that HES have an antifibrotic effects. These effects could be attributed to the action of HES as anti-fibrotic agents by decreasing the accumulation of ECM in the liver. HES in iron-intoxicated rats reduced the hepatic and renal histological changes induced by iron. This can be explained by the antioxidant and chelating ability of HES, which significantly decreased the oxidative stress, resulting in reduction of pathological changes and restoration of normal cellular functions.The protective effects were confirmed by histopathological examination and hepatic levels of the fibrotic. The results of the present study showed that; oral administration of HES (100 and 200 mg/kg) significantly improves hepatic architecture microscopically in a dose-dependent manner as the group of HES administration (100 mg/kg) shows slight improvement while the group of HES administration (200 mg/kg) show excellent improvement and slight difference with control normal group.

CONFLICTS OF INTEREST:

The author have declared no conflicts of interest

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