RESEARCH ARTICLE

Ameliorative Effects of Hesperidin on Piroxicam-Induced Haemato-Biochemical Alterations in Rats

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Abstract

Piroxicam, a non-steroidal anti-inflammatory drug, is widely prescribed in various inflammatory disorders. However, it causes side effects like hepato-renal toxicity, foetotoxicity and gastrointestinal toxicity etc. presumably due to production of oxidative stress. The bioflavonoid hesperidin, a flavanone glycoside, is reported to have many health benificial effects. The present study was designed to investigate the possible protective effects of hesperidin against piroxicam induced toxicity in male Wistar rats. Forty rats were divided into five groups with eight rats in each group. Groups I, II, III, IV and V were administered orally daily for 28 days with 1% carboxy methyl cellulose (CMC), piroxicam @ 10 mg/kg. b. wt., piroxicam @ 25 mg/kg. b. wt., piroxicam @10 mg/kg. b. wt. along with hesperidin @ 160 mg/kg. b. wt. and piroxicam @ 25 mg/kg. b. wt. along with hesperidin @ 160 mg/kg. b. wt., respectively. The results revealed that piroxicam @ 10 mg/kg b.wt. (Gr. II) caused no changes in haemato-biochemical parameters, while @ 25 mg/kg b. wt. (Gr. III) caused significant decrease in TEC, Hb, PCV, MCH, MCHC and platelet counts, and increase in TLC, neutophils and lymphocyte count in comparision to control Gr. I animals. Piroxicam caused significant increase in serum levels of liver and kidney function parameters including AST, total protein, BUN and creatinine as well as it also altered lipid metabolism @ 25 mg/kg b. wt.. The hesperidin was found to be effective against piroxicam induced pathological effects suggesting that hesperidin can be recommended to reduce the toxic effects produced by piroxicam.

Key words: Bioflavanoid, Hesperidin, NSAIDs, Oxidative stress, Piroxicam, Wistar rats.

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Introduction

Inflammation, is a biological phenomenon, response, a process, or a state of the system to perturbation (e.g., infection or injury), which is mediated as a multistep process of mobilizing defensive mechanisms to eliminate the source of perturbation, and it may result into an altered state of the system that can be either protective or pathological. In human and veterinary medicine, treatment of inflammation and pain is very important aspect. The non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed anti-inflammatory or pain medications. According to classification of NSAIDs based on their chemical structure, piroxicam is the first member of the oxicam or enolic acid derivatives, a nonspecific COX inhibitor and is prescribed for ankylosing spondylitis, musculoskeletal disorders, dysmenorrhea, acute gout and rheumatoid arthritis, various tumors like squamous cell carcinoma, mammary carcinoma, colorectal and invasive bladder cancers (Badawi, 2019; Abdeen *et al*., 2019). It is also reported that prolong use of piroxicam caused severe gastrointestinal toxicity, ulcerogenic gastropathy, renal hemostatic abnormalities, foetotoxicity, hepatotoxicity, nephrotoxicity, etc. (Ebaid *et al.,* 2007; Aithal, 2011). Since piroxicam is metabolized in the liver, there is a possibility of injury to the liver and piroxicam induced hepatic dysfunction and failure have also been reported (Sahu, 2016). The piroxicam mediated oxidative stress leads to lipid peroxidation (LPO) and free radical generation which in turn induces toxicity.

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Hesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone-7-rhamnoglucoside), a dietary flavanone is abundantly found in many citrus fruits and has a wide range of beneficial properties like anti-oxidative, anti-carcinogenic, anti-inflammatory, lipid-lowering activities, anti-allergic, anti-bacterial, anti-viral, neuroprotective and vascular protective (Yahia *et al.,* 2019; Mostafa *et al.,* 2023). The proteective effects of hesperidin against several drug induced toxicities have

been recorded. However, till date the ameliorative effects of hesperidin especially in piroxicam induced toxicity have not been studied. So the present work was planned to study the protective effects of hesperidin on piroxicam-induced haemato-biochemical alterations in Wistar rats.

Materials and Methods

Experimental Animals and Drugs

A total of 40 male Wistar rats weighing 200-350 gm were procured from Jai Research Foundation, Vapi, Gujarat. Rats were acclimatized for 10 days in the animal house of Veterinary College, KU, Navsari (India) before start of the experiment. During the experiment, the rats were housed in polypropylene cages and maintained under room temperature of 22±3°C with 12 h light and dark cycle and were provided with standard pelleted feed and water *ad libitum*. The experiment was approved by Institutional Animal Ethical Committee (IAEC) under the project no. 108-VCN-VPP-2022. The drug, piroxicam was procured from the Merck & Co., Inc, and Hesperidin from the Sigma-Aldrich company.

Experimental Design

Rats were randomly assigned into 5 groups of 8 rats each. Group I rats that received 1% Carboxy methyl cellulose (CMC) served as the vehicle control. Group II and III rats were injected with piroxicam at the dose rate of 10 mg/kg b. wt. and 25 mg/kg b. wt., respectively. Group IV and V rats received piroxicam @ 10 and 25 mg/kg b. wt., respectively, together with hesperidin @ 160 mg/kg b. wt. All compounds were administered once orally daily for 28 consecutive days.

Haemato-Biochemical Studies

Blood samples were taken from retro-orbital plexus using microcapillaries on 29th day of experiment in sterile vials containing K_3 EDTA for haematology and in plain vacutainers for serum biochemical analysis. An automatic Haemato-analyzer (Exigo EOS Vet, Sweeden) was used to evaluate haematological indices including platelet count, while DLC was done manually. Serum alanine aminotransferase (AST), aspartate aminotransferase (ALT), creatinine, BUN, total protein, albumin, cholesterol and triglycerides were determined using ready-to-use diagnostic kits with semi-automatic biochemistry analyser (Merck Instrument Model: Microlab 300, India).

Statistical Analysis

Data were subjected to statistical analysis using SPSS 20.0 statistical software (SPSS, Inc., 2009). One-way analysis of variance (ANOVA) followed by Duncan's test was performed to determine intergroup differences at $p<0.05$.

Results and Discussion

The results of the haematological and biochemical parameters observed in different experimental groups of rats are presented in Table 1 and 2, respectively.

Haematological Alterations

Data presented in Table 1 revealed that rats treated with piroxicam at dose rate of 10 mg/kg b. wt./day caused no significant changes in haematological parameters. While piroxicam at dose rate of 25 mg/kg b. wt./day caused significant ($p<0.05$) decrease in TEC, Hb, PCV, MCH, MCHC and platelets, and non-significant decrease in MCV values compared to control Group I. It was reported that piroxicam causes ulcer, bleeding, diffuse inflammation and increased mucosal permeability in gastrointestinal tract which might be the reason for decrease in TEC, MCV and Hb concentration, and development of microcytic hypochromic anaemia. In this study piroxicam induced thrombocytopenia. This result supported the previous findings (Vihol, 2010). The NSAID like aspirin stimulates the production of the platelet inhibitor nitric oxide (NO) *in vitro* and *in vivo,* which may contribute to its antiplatelet activity leading to thrombocytopenia. In the high dose group III of piroxicam leukocytosis, neutrophilia and lymphopenia were noted suggesting inflammatory response induced by piroxicam toxicity. The cyclooxigenase I enzyme is useful for the stomach mucosal intergrity by enhancing bicarbonate ions secretions. The drug piroxicam is nonselective COX 1 inhibitor. Inhibition of this enzyme leads to blockage of bicarbonate ion secretions that leads to increased pH of stomach mucosa and formation of ulcer. Along with ulcer formation inflammatory response also increases which ultimately results in increased leucocyte counts. In acute inflammation, number of neutrophils increases and these might be possible for increased leucocyte and neutrophil counts in present study (Vihol, 2010).

On the other hand, group V treated with hesperidin at dose rate of 160 mg/kg b. wt./day along with piroxicam @ 25 mg/kg b.wt./day showed non-significant increase in values of TEC, Hb, PCV, MCV, MCH, MCHC and platelet counts, and decrease in TLC, neutophils and lymphocyte count in comparison to group III administerd with high dose of piroxicam @ 25 mg/kg b.wt./day alone. In group IV treated with hesperidin @ 160 mg/kg b. wt./day along with piroxicam @ 10 mg/kg b.wt./day, haematological values were comparable with control group I and even group II treated with piroxicam @ 10 mg/kg b.wt./day alone. Hesperidin works as hydrogen-donor and free-radical scavenger as well as it acts as a potential chain breaking antioxidant and it also chelates transition of metal ions, hence can inhibit free radical formation and the propagation of free-radical reactions (Mohamed *et al*., 2015). Hesperidin improves the erythrocyte membrane integrity by reduction of oxidative damage to the erythrocyte's membrane (Afolabi *et al.,* 2019).

Serum Biochemical Alterations

Data presented in Table 2 revealed that, the concentration of AST, triglyceride, BUN and creatinine increased significantly

Table 1 : Details of haematological values of different treatment groups of rats (Mean ± SE)

Parameters	Group I (N=8)	Group II (N=8)	Group III (N=8)	Group IV (N=8)	Group V (N=8)
TEC (x 10^6/µL)	7.81^{b}±0.11	7.76^{b}±0.11	6.62^{a}±0.30	7.78^{b}±0.15	7.03^{ab}±0.41
Hb (g/dL)	15.48^{b}±0.28	15.20^{b}±0.22	12.70^{a}±1.01	15.38^{b}±0.93	14.20^{ab}±0.42
PCV (%)	42.30^{b}±0.75	41.75^{b}±0.41	35.39^{a}±1.14	42.17^{b}±0.79	39.27^{b}±0.93
MCV (fL)	54.20±0.49	53.12±0.61	51.56±0.82	54.56±0.45	53.66±0.94
MCH (pg)	19.22^{b}±0.22	18.63^{ab}±0.46	16.11^{a}±0.82	19.03^{b}±0.70	18.18^{ab}±0.44
MCHC (g/dL)	36.12^{b}±0.19	34.42^{ab}±1.19	32.11^{a}±1.13	35.37^{b}±1.36	34.68^{ab}±0.88
TLC (x 10^3/µL)	8.21^{c}±0.26	8.39^{bc}±0.53	10.06^{a}±0.36	8.14^{bc}±0.27	8.65^{ab}±0.28
Lymphocyte (%)	70.62^{b}±1.75	68.25^{ab}±1.17	65.00^{a}±1.87	69.75^{b}±1.26	68.25^{ab}±1.41
Neutrophils (%)	23.50^{a}±1.75	25.00^{ab}±1.00	29.00^{b}±1.87	24.87^{ab}±1.05	27.50^{ab}±1.25
Eosinophils (%)	1.25±0.25	1.37±0.18	1.50±0.18	1.25±0.25	1.37±0.26
Monocyte (%)	4.62±0.49	4.37±0.46	4.50±0.37	4.12±0.47	4.22±0.22
Platelets (x 10^3/µL)	844.25^{ab}±27.02	792.62^{ab}±24.73	770.75^{c}±27.07	802.75^{a}±24.57	795.12^{bc}±24.50

N = Number of animals, Means bearing different superscripts differ significantly (p<0.05) between groups.

Table 2 : Serum biochemical values reported in different experimental groups (Mean ± SE)

Parameters	Group I (N=8)	Group II (N=8)	Group III (N=8)	Group IV (N=8)	Group V (N=8)
AST (U/L)	106.53^{a}±11.41	125.38^{ab}±12.09	$145.90^{b\pm}$13.67	$108.45^{a\pm}$15.68	110.45^{a}±18.83
ALT (U/L)	34.92±1.66	37.47±2.13	43.15±3.24	35.95±1.93	37.10±2.11
Total protein (g/dL)	7.12^{b}±0.27	6.67^{b}±0.19	5.61^{a}±0.17	7.06^{b}±0.20	6.60^{b} ±0.16
Albumin (g/dL)	4.02 ±0.39	3.66±0.25	2.27±0.23	3.87±0.17	3.72±0.13
Triglyceride (mg/dL)	129.35^{b}±5.77	130.82^{ab}±2.28	147.30^{a}±8.29	120.34^{ab}±4.22	135.66^{ab}±3.66
Cholesterol (mg/dL)	100.46±5.04	118.84±5.69	130.12±4.60	114.43±4.28	123.34±5.12
BUN (mg/dL)	19.55^{ab}±1.13	20.38^{abc}±1.20	24.25^{c}±0.67	17.14^{a}±0.99	22.14^{bc}±2.31
Creatinine (mg/dL)	0.75^{a}±0.16	0.91^{c}±0.53	1.02^{c}±0.10	0.72^{ab}±0.03	0.88^{bc}±0.11

N = Number of animals, Means bearing different superscripts between groups differ significantly (P<0.05).

(p<0.05) and total protein decresed significantly in rats of group III treated with piroxicam @ 25 mg/kg b. wt./day alone. Moreover, non significant increase in concentration of ALT and cholesterol, and decrease in albumin values were noted in this group III as comparesd to control group I. In groups II, IV and V non-significant increase in concentration of AST, ALT, triglyceride, cholesterol and BUN and decrease in total protein and albumin were noted. The creatinine value did not differ significantly among groups I, II and IV. The alteration in liver enzymes, *viz*., AST and ALT in group III suggest that piroxicam at dose rate of 25 mg/kg b. wt./day induced liver dysfunction, while hesperidin at dose rate of 160 mg/kg b. wt./day nullified the toxic effects produced by high dose of piroxicam. Piroxicam damages the membrane integrity by inducing oxidative stress in liver and these might have caused seeping out of AST and ALT enzymes from liver. Hesperidin acts as a potent anti-oxidant agent and hence decreases the production of reactive oxygen species (ROS). Moreover, it also stabilizes the hepatic cellular membrane damage and protects the hepatocytes against the toxic substances which might decrease the leakage of the enzymes into blood stream (Majeed and Abass, 2017).

The total protein value (5.61±0.17 g/dL) in group treated with piroxicam at high dose (25 mg/kg b. wt./day) was significantly lower, while albumin showed non-significant decrease and this indicates altered protein metabolism in animals. The enhanced generation of ROS, damages mitochondria, the endoplasmic reticulum and DNA which results in altered protein synthesis that leads to inhibition of gene expression and mRNA translation, protein unfolding and aggregation of damaged proteins. These ROS induced damage causes massive necrosis of liver and disturbance in liver function, as indicated by considerable decline in serum albumin and consequently, total protein content in our study in piroxicam groups III and II. Impaired tubular reabsorption might be another possible reason for increased protein loss (Abdeen *et al*., 2019). Hesperidin treatment improved enzymatic activities, total protein and albumin which indicates its protective effect (Elshazly and Mahmoud, 2014; Kamel *et al*., 2014).

Altered values of triglyceride (147.30±8.29 mg/dL) and cholesterol (130.12±4.60 mg/dL) indicate altered lipid metabolism in group III. The significantly (p<0.05) higher values of BUN (24.25±0.67 mg/dL) and creatinine (1.02±0.10 mg/dL) levels showed that piroxicam induced renal injury at dose of 25 mg/kg b.wt./day. In hesperidin co-administered groups IV and V, restoration in serum values of AST, ALT, triglyceride, cholesterol, BUN, total protein and albumin were noted suggesting ameliorative effect of hesperidin on liver

and kidney toxicities. In this study, the level of cholesterol and triglycerides were found to be increased in piroxicam treated group and decreaded in hesperidin treated groups. Liver plays major role in synthesis and controlling the cholesterol and triglycerides. Piroxicam's adverse effects cause impairment in lipid metabolism by producing ROS. Free radicals such as superoxide anion, hydrogen peroxide and hydroxyl radical damage the lipid components of the cell membrane by peroxidation and denaturation of proteins, which leads to change in the levels of cholesterol and triglycerides (Abdeen *et al.,* 2019). It is believed that the antioxidant property could also play a major role in protection of lipid membranes from free radicals. Hesperidin attenuates the abnormal dispersion of membrane lipids in circulation as well as reduces the excessive generation of more toxic lipid peroxides which results in less harmful effects on cells and tissues (Pari *et al*., 2015).

Present result indicated increase in BUN and creatinine in piroxicam treated group which indicated renal injury caused by piroxicam. It is reported that piroxicam causes renal injury in the experimental animals mostly by damaging the PCTs (Majeed and Abass, 2017). Increased creatinine indicates altered GFR in affected animals and reason for this might be the oxidative stress induced by generation of ROS through metabolic activation of highly reactive free radicals (including superoxidase and ROS) in piroxicam toxicity. Hesperidin has potent anti-oxidant capacity so it reduces the production of ROS which in turn helps in maintaining the normal level of GFR and ultimately decreases the serum creatinine and BUN levels (Sorathiya, 2021). It is reported that hesperidin (HES) acts by scavenging free radicals and by maintaining intracellular superoxide dismutase (SOD) and glutathione levels, thereby prevents lipid peroxidation and tissue damage.

Conclusions

The findings of the present study revealed that piroxicam at dose rate of 10 mg/kg b. wt. produced no observable alteration in haemato-biochemical parameters, whereas piroxicam at dose rate of 25 mg/kg b. wt. caused haemato-toxicity, hepatotoxicity, nephrotoxicity, GIT toxicity, and dyslipidemia in rats. Moreover, hesperidin co-administration revealed excellent antioxidant activity with haemato-protective, anti-lipidemic, hepato-protective, GIT-protective and nephro-protective effects against organ toxicities caused by piroxicam at both the dose rates.

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