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Original Article

Sub-chronic toxicological studies of transition metal complexes of naproxen on sprague-dawley rats

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ABSTRACT

Objective: The purpose of this research was to investigate sub-chronic toxicity in animal model.
Methods: A detailed study was done on the physical, hematological, biochemical and hormonal parameters of both male and female Sprague-Dawley rats after 28 days administration of naproxen and its metal complexes.
Results: There were no significant changes found in physical parameters on observation for both male and female rats without some minor differences. However, Naproxen metal complexes showed comparatively lower side effects than naproxen. Hematological report suggested that naproxen was in process of initiating inflammation which was justified by decreasing the mean value hemoglobin and hematocrit level and increasing the white blood cells level. There were no significant changes in biochemical parameters, however, the mean value of blood glucose level and cholesterol seemed to be higher and triglyceride was lower. Thyroid hormone levels were found lower, that was another indication inflammatory process. However, this might have the ability to lower the insulin secretion resulting in increasing blood glucose level.
Conclusion: In the present investigation, there were no significant alterations in histopathological studies and physical parameters though some signs of abnormalities had been found but hematological and hormonal data did not suggest any inflammatory or toxicological activity. However, we observed that naproxen showed more side effects than metal complexes which indicated that carboxylic group (—COOH) of naproxen may be responsible for showing those most of the side effects.

1. Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely utilized classes of drugs due to their potent anti-inflammatory, analgesic, and anti-pyretic properties in the world.[1] The treatment of inflammation and pain is an important area of therapeutics. In the last decade, nonsteroidal anti-inflammatory drugs (NSAIDs) like naproxen have played a central role in these indications and they are currently considered as the first choice, being one of the most widely prescribed drugs.[2,3] Naproxen is a non steroidal anti-inflammatory, analgesic drug which is extensively used in the clinical treatment of acute and chronic pain and arthritis.[4] They inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes involved in the synthesis of prostaglandins.[5] Prostaglandins are chemicals produced by the cells of the body promoting inflammation, pain and fever; supporting the blood clotting function of platelets and protecting stomach from the damaging effects of acid.[6,7] Cyclooxygenase-1 is constitutively expressed and generates prostanoids involved in the maintenance of the integrity of gastrointestinal mucosa and platelet aggregation[8] whereas at sites of inflammation, cyclooxygenase-2 is induced to generate prostaglandins that mediate inflammation and pain.[9] They are highly effective for their anti-inflammatory and analgesic properties in treating different level of pains, such as osteoarthritis and rheumatoid arthritis[10] and may reduce the risk of colon cancer and probably various types of gastrointestinal (GI)-related cancers.[11] Despite all of those successes, many studies reveal that gastric or duodenal ulcers develop in 15–30 percent of patients who regularly take NSAIDs[12] and more than 100,000 patients are hospitalized and 16,500 die every year in America as a result of NSAID induced gastrointestinal events.[13,14]

As there is no information available on the sub-chronic toxicological effects of metal complexes of naproxen in the animal model compared to naproxen, we have motivated to study metal binding properties of naproxen with different transition metal ions with the aims of investigating their physical, hematological,

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biochemical and hormonal effects on the body by evaluating acute toxicity in animal model. In this paper, we report the synthesis of some organ metallic compounds of naproxen and their ability to reduce gastrointestinal toxicity.

2. Materials and methods

2.1. General procedure for synthesis of transition metal complexes of naproxen

All synthetic procedures were described in details by Hasan et al.[15]

2.2. Method consideration, dose selection and route of administration

The sub-chronic model was used to evaluate NSAID induced pathological state in young, healthy rat model to see overall effect on the health of the animals over the 28 days dosing period. The sub-chronic model allows one to assess the test NSAID's toxicity with regard to GI bleeding (hemoglobin, hematocrit reduction), the development of intestinal perforation and adhesions.[16] The dose of naproxen employed in the rat studies was equivalent to 10 mg/kg body weight. This dose was selected because it produced significant and comparable activity in reducing paw swelling in rats with adjuvant arthritis and also producing several side effects including ulceration.[17] Using this dose, other naproxen metal complexes are also administered at a dose of 10 mg/kg and test samples were orally administered twice daily for 18 days.[18]

2.3. Experimental design

All experimental animals (Supplementary Sections 1.1 and 1.2) were randomly selected and divided into seven groups in Table 1.

2.4. Preparation of test samples

The calculated amount of the test samples were measured and normal saline was added with 1–2 drops of a suspending agent. To stabilize the suspension, it was stirred well by vortex mixture. Finally the volume was adjusted up to such so as to have final volume with concentration vol-dose/group/2 administrations per day.[18]

2.5. Sacrificing of the animals and methods of observation and examination

At the end of treatment period (28 days), the animals were sacrificed, physical observation was performed and blood samples and organs were collected for further experiments (Supplementary Sections 1.3, 1.4 and 1.5).

2.6. Histopathologic studies

2.6.1. Microscopic evaluation of histopathological study

The stomach was opened along with greater curvature, rinsed with saline to remove gastric contents and blood clots using washout technique[19,20] and examined by a 5× magnifying lens to assess the formation of ulcers. The stomach was removed and the number of ulcers was counted.

2.6.2. Measurement of Ulcer score

Ulcer index was measured by using following formula.[19] Ulcer scoring was described in Table 2.

$$UI = UN + US + UP \times 10^{-1}$$

UI = Ulcer Index.
UN = Average number of ulcers per animal.
US = Average number of severity score.
UP = Ulcer probability (incidence%) for each group.

Percentage inhibition of ulceration was calculated as below:

$$\%\ \text{Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{\text{Standard}} - \text{Ulcer index}_{\text{Test compound}}) \times 100}{(\text{Ulcer index}_{\text{Standard}})}$$

2.7. Statistical analysis

All the grouped data were statistically evaluated with SPSS version 17 software. All the results were expressed as Mean ± SEM (Standard Error of Mean) values for seven animals in each group. Means were compared by two tailed independent sample *t*-test. Probability (p) value of 0.05 or less ($p < 0.05$) was considered as significant.

3. Results

3.1. Characterization of metal complexes of naproxen

Physical, analytical and thermal properties, NMR spectra, FTIR spectra, scanning electron microscopy and HPLC study of naproxen metal complexes were described by Hasan et al.[15]

3.2. Toxicological evaluation

3.2.1. Physical parameters

No significant changes were observed in both male and female rats without some minor alterations within any sample groups throughout the dosing period. No mortality was observed in all groups of both male and female rats during the experiment (see Fig. 1).

3.2.2. Weight variation of rats during the study period

In an attempt to observe any change in the body weight of the tested animals induced by naproxen and its metal complexes, the

Table 1
Experimental design of naproxen metal complexes.

Groups	Test samples	No of animals
Group I	Control	8
Group II	Naproxen	8
Group III	Naproxen copper complex	8
Group IV	Naproxen cobalt complex	8
Group V	Naproxen iron complex	8
Group VI	Naproxen silver complex	8
Group VII	Naproxen zinc complex	8

Table 2
The number of ulcers was counted. Ulcer scoring was undertaken21 as following manner.

Ulcer score	Descriptive/observation
0	No ulcer/normal colored stomach
0.5	Red coloration
1.0	Superficial (spot) ulcer
1.5	Hemorrhagic streak
2.0	Deep ulcer
3.0	Perforation