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A Detailed Study on Acute Toxicity of Transition Metal Complexes of Naproxen

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Abstract

The objective of this research was to investigate the acute toxicity of naproxen metal complexes in animal model. A detailed study was done on the physical, biochemical and hormonal parameters of both male and female swiss-albino mice after 14day administration of naproxen and its metal complexes. There were no significant changes found in physical parameters observation for both male and female mice without some minor differences. However, naproxen metal complexes showed comparatively lower side effects than naproxen. This might indicated that the carboxylic group of naproxen(-COOH) took part in pathological reactions resulting in side effects in different parts of the organ. 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. We did not find any changes in sex hormones level in this study.

Keywords: Naproxen, metal complex, acute toxicity

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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]Naproxen is a nonsteroidal anti-inflammatory analgesic drug which is extensively used in the clinical treatment of acute and chronic pain and arthritis.[2]They inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes involved in the synthesis of prostaglandins.[3]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.Cyclooxygenase-1 is constitutively expressed and generates prostanoids involved in the maintenance of the integrity of gastrointestinal mucosa and platelet aggregation whereas at sites of inflammation, cyclooxygenase-2 is induced to generate prostaglandins that mediate inflammation and pain.They are highly effective for their anti-inflammatory and analgesic properties in treating different level of pains, such as osteoarthritis and rheumatoid arthritis and may reduce the risk of colon cancer and probablyvarious types of gastrointestinal (GI)-related cancers. Despite all of those successes, many studies reveal that gastric or duodenal ulcers develop in 15 to 30 percent of patients who regularly take NSAIDsand more than 100,000 patients are hospitalized and 16,500 die everyyear in America as a result of NSAID induced gastrointestinal events.[4]

In case of Naproxen, gastrointestinal side effects are the most common occurrence including constipation (3% to 9%), general abdominal discomfort (3% to 9%), nausea (3% to 9%), dyspepsia (3% to 9%), diarrhea, and stomatitis. Serious gastrointestinal side effects include peptic ulcerations, gastrointestinal perforation etc.Abnormalities in the central nervous system are depression, insomnia, muscle weakness, aseptic meningitis and cognitive disorder. Those who have

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reduced renal function may be at increased risk for renal side effects like acute renal failure, papillary necrosis, acute tubular necrosis etc.Other problems like cardiovascular side effects, hematological effectsand respiratory effects are also very common for naproxen.

The use of metal complexes as therapeutic agents for treatment of different diseases has been extensively studied.[5]Our previous work showed detailed explanation about pharmacological properties of naproxen metal complexes.[6]Many researchers have proved that binding of a drug to metalloelement enhances its activity and in many cases the complex posses such activity that can reduce or even eliminate the side effects of the parent compound.[7]As there is no information available on the acute toxicological effects of metal complexes of naproxen in the animal model compared to naproxen, we have motivated to study with the aims of investigating their physical, hematological, biochemical and hormonal effects on the body by evaluating acute toxicity in animal model.

2. Materials and Methods

Procedure for the synthesis of transition metal complexes of naproxen

A detailed procedure of synthesis of transition metal complexes were described inour previous paper.[8]

Method consideration and experimental design

The acute toxicity study was performed to evaluate naproxen induced pathological stage in young, healthy mice model to see overall toxicity effects after 14 days. Selection of experimental animals, sample preparation,dose selection and route of administration, sacrificing of the animalsafter 14th day andmethodology of blood sample collectionwere described in our previous paper.[9]

Methods of observation and examination

Physical observations

Animals were observed daily for a total of 14 days. Observations included changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, activities of nervous systems, somatomotor activity and behavior pattern. Attention was given to tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Other physical parameters like dizziness, drowsiness, fluid retention, swelling, changes in urine/stool color, itching, skin rash, body weight, local injury were also studied during treatment of animals. No mortality was found during the treatment of all groups.

Biochemical and hormonal parameters

The serum separated was analyzed by Star 21 Plus auto analyzer using the commercially available standard kit (ERBA Diagnostics Mannheim GmbH, Mannheim, Germany) for determination of various parameters such as creatinine, urea, uric acid, total serum protein, blood urea nitrogen (BUN), bilirubin, albumin, blood sugar levels, cholesterol, triglycerides, AST (aspartate aminotransferase, also known as serum glutamic oxaloacetic transaminase (SGOT)), ALT (alanine aminotransferase, also known as serum glutamic pyruvic transaminase activities (SGPT)) and alkphos (alkaline phosphatase).

The hormonal analyses were performed by using the commercially available kits and according to the instructions of the manufacturer for Radio Immuno Assay technique. We assayed free T3 (FT3), free T4 (FT4), TSH, T4, T3, Testosterone and Progesterone because if there were any physical parameters alterations seen, we would need to focus the hormonal level of thyroid and sex hormones as they regulate many influential effects on physiological activity and might have any influences on either inflammation or other inflammatory response activity.

3. 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 eight 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.

4. Results

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.[8]

Toxicological evaluation

Weight variation of mice 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 weights were taken at initial period (0 day), 7th day and 14th day of study. The alteration of the body weight of the experimental animals was not so prominent. No significant changes were observed.

Physical parameters

No significant changes were observed in both male and female mice without some minor alterations within any sample groups throughout the dosing period [Table 1] and [Table 2]. However, there were few abnormalities found in female mice which were indicated in bold letters. No mortality was observed in all groups of both male and female mice during the experiment.

Table 1. Physical parameter changes of male mice during treatment of test compounds

Parameters		Naproxen	N-Cu	N-Co	N-Fe	N-Ag	N-Zn
Changes in skin, eye and fur		Not found	Not found	Not found	Not found	Not found	Not found
Behavior pattern		No alterations	No alterations	No alterations	No alterations	No alterations	No alterations
Tremors, convulsions		Not found	Not found	Not found	Not found	Not found	Not found
Diarrhea/ Constipation		Constipation observed	Not found	Not found	Constipation observed	Constipation observed	Not found
Salivation		Not found	Not found	Not found	Not found	Not found	Not found
lethargy, sleeping behavior and coma	Day	Seemed to be sleepy	Sleepy	Sleepy	Sleepy	Sleepy	Sleepy
	Night	Not done	Not done	Not done	Not done	Not done	Not done
Nausea and vomiting		Not found	Not found	Not found	Not found	Not found	Not found
Headache/ Dizziness/ Drowsiness		No	No	No	No	No	No
Fluid retention/ Swelling		No	No	No	No	No	No
Itching/ Skin rash		No	No	No	No	No	No
Changes in urine/stool color		Light blackish stool	No	No	No	No	No

Table 2. Physical parameter changes of female mice during treatment of test compounds

Parameters		Naproxen	N-Cu	N-Co	N-Fe	N-Ag	N-Zn
Changes in skin, eye and fur		Not found	Not found	Not found	Not found	Not found	Not found
Behavior pattern		No alterations	No alterations	No alterations	No alterations	No alterations	No alterations
Tremors, convulsions		Not found	Not found	Not found	Not found	Not found	Not found
Diarrhea/ Constipation		Constipation may occur	Not found	Not found	Constipation observed	Not found	Not found
Salivation		Not found	Not found	Not found	Not found	Not found	Not found
lethargy, sleeping behavior and coma	Day	Seemed to be sleepy	Sleepy	Sleepy	Sleepy	Sleepy	Sleepy
	Night	Not done	Not done	Not done	Not done	Not done	Not done
Nausea and vomiting		Not found	Not found	Not found	Not found	Not found	Not found
Headache/ Dizziness/ Drowsiness		No	No	No	No	No	No
Fluid retention/ Swelling		No	No	No	No	No	No
Itching/ Skin rash		Yes	Yes	No	Yes	No	No
Changes in urine/stool color		Light blackish stool	No	No	No	No	No

Biochemical Assessment

All the biochemical parameters studied was found to be comparable with control group [Table 3] and [Table 4]. In both male and female mice groups, no significant changes were seen in any parameters. However, the blood glucose and cholesterollevel seemed to be higher and triglyceride was found lower for naproxen treated groups.

Hormonal Assessment

No significant results have been obtained from investigations on the influence of thyroid gland on inflammation [Table 5] and [Table 6].However, naproxen treated animals showed comparatively reduced thyroid level than other metal complexes. Many previous research works suggested that thyroid gland activity could influence the inhibition of inflammatory reactions [10], [11] and sometimes it looked most likely to be indirect. [11]

Sex hormones both testosterone and progesterone level were found normal in all treated groups compared to control. However, a long term study is necessary for getting a better activity of those hormones on inflammation.

Table 3. Biochemical parameter changes of male mice during treatment of test compounds

Parameters	Control	Naproxen	N-Cu	N-Co	N-Fe	N-Ag	N-Zn
Glucose (mg/dl)	112.7 ± 3.1	114 ± 4.1	110± 2.7	114 ± 1.7	111 ± 2.9	109 ± 3.1	113 ± 1.4
BUN (mg/dl)	38.8 ± 2.9	36.5 ± 2.1	38.3± 4.1	34.9 ± 3.8	39.4± 2.5	37.3 ±4.2	35.7± 2.3
Creatinine (mg/dl)	1.2 ± 0.45	1.3 ± 0.57	1.6 ± 1.0	1.2 ± 0.88	0.9 ± 0.69	1.5 ± 0.24	1.4 ± 0.63
Protein (g/L)	44.8 ± 6.3	47.6 ± 7.3	41.4± 3.6	49.7± 8.2	46 .2± 8.1	43.5± 5.7	48.3 ± 4.1
Albumin (g/L)	45.4 ± 7.0	43.8 ± 7.0	47.4 ± 7.0	44.2± 7.0	45.5± 7.0	44.3± 7.0	42.7± 7.0
Total cholesterol (mg/dl)	115.8± 9.3	117.7± 11.3	116.3± 8.3	116.7± 10.4	115.3± 7.2	114.9± 6.6	117.0± 6.4
Triglycerides (mg/dl)	95.6 ± 8.5	97.8 ± 6.3	94.1 ± 7.5	94.9 ± 6.9	96.9 ± 9.1	94.6 ± 7.9	91.6 ± 9.5
Total bilirubin (mg/dl)	0.5 ± 0.2	0.3 ± 0.4	0.2 ± 0.5	0.5± 0.6	0.5 ± 0.7	0.4 ± 0.3	0.5 ± 0.4
Urea (mg/dl)	4.37±0.35	5.07±0.25	5.61±0.43	4.67±0.77	3.67±0.89	3.45±0.95	5.27±0.45
Uric acid (mg/dl)	3.21±0.8	3.35±0.9	2.93±0.6	3.74±0.7	3.82±0.6	3.69±0.9	2.86±0.8
ALT (IU/L)	95.6 ± 8.3	99.4 ± 5.3	100.3±6.7	95.8 ± 7.3	98.2± 5.9	96.5± 8.7	97±8.1
AST (IU/L)	193± 10.6	196.3± 8.6	194.6± 9.8	195.2± 7.6	191.4± 8.9	196.3± 9.9	195± 11.1
Alkphos (IU/L)	40.5± 2.7	42.8± 2.9	44.5± 4.7	38.5± 1.7	41.8± 3.4	43.1± 1.3	42.9± 4.1

Data were expressed as mean±SEM. n=8, p<0.01 compared to control.

Table 4. Biochemical parameter changes of female mice during treatment of test compounds

Parameters	Control	Naproxen	N-Cu	N-Co	N-Fe	N-Ag	N-Zn
Glucose (mg/dl)	114.3 ± 2.7	115.6 ± 3.1	113.5± 2.1	113.2 ± 1.2	114.3 ± 2.1	113.9 ± 3.2	113.8 ± 1.3
BUN (mg/dl)	37.4 ± 1.9	37.7± 1.1	37.5± 2.0	37.2 ± 0.3	36.9± 1.7	36.7 ±2.1	36.7± 1.1
Creatinine (mg/dl)	1.1 ± 0.65	1.0 ± 0.43	1.3 ± 1.2	1.2 ± 0.48	1.1 ± 0.79	1.2± 0.54	1.4 ± 0.33
Protein (g/L)	46.2 ± 2.2	47.4 ± 2.3	46.8± 1.7	47.7± 3.0	46 .5± 2.2	45.3± 2.8	45.4 ± 2.1
Albumin (g/L)	44.8 ± 2.1	43.9 ± 3.7	45.1 ± 2.5	45.4± 3.0	45.3± 2.8	45.3± 3.1	44.7± 1.7
Total cholesterol (mg/dl)	116.7± 4.6	118.4± 5.2	115.6± 4.3	117.7± 4.9	116.2± 4.8	115.4± 5.6	117.1± 5.8
Triglycerides (mg/dl)	96.2 ± 3.6	94.7 ± 4.5	97.1 ± 4.1	95.9 ± 3.9	97.6 ± 5.3	95.6 ± 4.9	96.6 ± 5.5
Total bilirubin (mg/dl)	0.6 ± 0.3	0.5 ± 0.3	0.7± 0.2	0.5± 0.3	0.6 ± 0.4	0.6 ± 0.7	0.5 ± 0.4
Urea (mg/dl)	4.56±0.25	5.07±0.55	4.61±0.63	4.47±0.47	5.10±0.76	4.45±0.65	5.01±0.45
Uric acid (mg/dl)	3.45±0.8	3.35±0.9	3.53±0.8	3.24±0.8	3.32±0.5	3.29±0.3	3.46±0.7
ALT (IU/L)	97.4 ± 3.3	98.4 ± 3.3	96.8±2.7	97.8 ± 5.3	97.2± 3.9	96.9± 3.5	98.2±4.1
AST (IU/L)	195.3± 7.7	194.3± 8.9	196.2± 8.8	195.6± 6.9	196.4± 7.4	194.8± 5.9	195.2± 9.1
Alkphos (IU/L)	42.2± 2.2	43.1± 3.0	41.5± 1.7	41.5± 1.9	42.8± 2.4	43.1± 2.3	42.9± 2.8

Data were expressed as mean±SEM. n=8, p<0.01 compared to control.

Table 5. Hormonal parameter changes of male mice during treatment of test compounds

Parameters	Control	Naproxen	N-Cu	N-Co	N-Fe	N-Ag	N-Zn
Free T_3 pg/mL	2.19±0.112	2.13±0.263	2.31±0.178	2.29±0.235	2.36±0.157	2.18±0.232	2.25±0.247
Free T_4 ng/dL	0.40±0.142	0.42±0.121	0.49±0.253	0.35±0.242	0.32±0.013	0.36±0.021	0.41±0.211
TSH, mU/liter	27.4 ±2.6	25.3 ±3.0	28.2± 3.4	26.5 ±2.4	22.4 ±3.4	29.8± 1.5	24.9 ±3.1
T_4, mg/dl	3.81 ± 0.88	3.68 ±0.63	3.85 ±0.48	3.87 ±0.51	3.79 ±0.64	3.77 ±0.58	3.86 ±0.48
T_3, ng/dl	85.8 ± 3.6	83.2 ± 3.1	84.8 ± 4.1	83.9 ± 2.9	86.2 ± 2.8	83.2 ± 3.1	88.3 ± 3.6
Testosterone (male)mg/ml	11.4± 2.4	10.4± 2.4	10.8± 0.7	12.4± 2.9	10.9± 2.4	10.7± 1.5	10.4± 1.7

Data were expressed as mean±SEM. n=8, p<0.01 compared to control.

Table 6. Hormonal parameter changes of female mice during treatment of test compounds

Parameters	Control	Naproxen	N-Cu	N-Co	N-Fe	N-Ag	N-Zn
Free T_3 pg/mL	2.24±0.21	2.11±0.23	2.35±0.17	2.19±0.27	2.29±0.28	2.31±0.24	2.23±0.21
Free T_4 ng/dL	0.45±0.19	0.41±0.12	0.48±0.27	0.39±0.31	0.37±0.30	0.42±0.29	0.46±0.21
TSH, mU/liter	27.3 ± 2.2	24.6 ± 2.8	28.4 ± 3.2	26.5 ± 1.7	29.1 ± 3.8	28.4± 2.4	26.6 ± 3.3
T_4, mg/dl	3.96 ±0.25	3.72 ±0.32	3.89 ±0.39	3.91 ±0.36	3.85 ±0.34	3.90 ±0.41	3.89 ±0.36
T_3, ng/dl	83.1 ± 2.9	80.2 ± 2.7	82.4 ± 2.3	84.2 ± 3.1	84.5 ± 2.6	82.7 ± 3.2	85.1 ± 3.9
Progesterone (mmol/l)	39.3± 3.2	41.2± 3.2	38.3± 3.1	40.7± 3.2	39.8± 2.2	39.1± 3.7	38.8± 1.9

Data were expressed as mean±SEM. n=8, p<0.01 compared to control.

5. Discussion

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 that indicated that carboxylic group (-COOH) of naproxen may be responsible for showing those most of the side effects. Based on this result we strongly believe that –COOH group of naproxen predominantly responsible for showing side effects in various compartments of the body. Hence metal complexes lack the –COOH group because of complexation with metals they did not show any considerable side effects than naproxen. No any major changes in behavior or tremors/convulsions or body weight were seen during the study period.

Biochemical parameters related to kidney function were found normal for all the samples. However, glucose level of the naproxen treated animals showed higher value than control and other samples. We are not sure whether this was a sign of abnormality or not hence the mean value is higher but statistically not significant. If yes then we must take the consideration of hormonal level of that group.

In case of hormonal parameters evaluation we observed that thyroid hormone level of naproxen treated group was slightly lower than control group. Other groups had normal thyroid level. Lowered thyroid level can influence the development of inflammatory reactions whereas excess of circulating thyroid hormones may depress inflammatory responses.[10],[11]Moreover, lower thyroid hormones may also have influence of increasing the blood glucose level and ketone bodies[12] as it was reported that these hormones induced insulin secretion in rats.[13]As we are not sure why the thyroid hormone level is altered in naproxen groups,it would be better if we knew the cortisol level(the "stress" hormone)of that group.Reduced level of thyroid hormone sometimes indicates an excess level of cortisol in the body. If the cortisol level was higher than we concluded that there are some effects for reducing the thyroid hormones level that led to increased glucose level in blood. If the cortisol level was in normal value then long term sub-chronic or chronic toxicity study should be performed for getting a better idea about hormonal secretion activity during naproxen and metal complexes therapy. Alterations of sex hormones level were sometimes predicted responsible for inflammatory actions, however, we got testosterone and progesterone hormones in normal range. We believe that a clear understanding can be found in the long term studies on effects of hormonal secretion and activity on

toxicity or inflammation during naproxen and metal complexes administration.

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7. Conflicts of interest

We declare no conflict of interest.

8. References

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