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Iron oxide magnetic nanoparticles in NSAID-induced gastrointestinal side effects: a possible new approach in pain treatment

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Abstract: Non-steroidal antiinflammatory drugs are widely used mostly for pain treatment, but are correlated with several side effects, especially gastrointestinal and cardiovascular. Recently, different types of nanoparticles were reported to succesfully treat various pathologies, with fewer side effects than the active substance alone. In this context, the aim of the present research was to study the gastrointestinal side effects of iron oxide magnetic nanoparticles functionalized with ibuprofen (IMNPs), compared to ibuprofen alone; 30 Wistar white rats, divided into 3 equal groups (5 female/group, 5 male/group), received, daily, for 10 consequitive days, oral treatment with IMNPs or ibuprofen alone. The effects of the treatment were assessed regarding oxidative stress parameters (at serum and gastric level) and hepatic function by dosing the ASAT and ALAT parameters at the beginning of the study (before treatment) and, respectively after 10 days of treatment; ASAT values showed increased liver damage for both treatment groups, but with no statistically significant differences between the two groups. The ALAT values were statistically lower for the IMNPs group compared to the ibuprofen group. IMNPs appear to have an antioxidant action compared to the control group or ibuprofen alone, regarding the GSH and GSH/GSSG levels; Ibuprofen functionalized iron oxide magnetic nanoparticles seem to reduce the side effects of ibuprofen at serum and gastric level, representing a potential alternative for pain treatment, but more studies are needed in this direction.

Keywords: nanoparticles, ibuprofen, oxidative stress

1. Introduction

Nonsteroidal anti-inflammatory (NSAIDs) medication is widely used in pain treatment, but, as well known, they have several side effects, both gastrointestinal and cardiovascular. Globally, NSAIDs prescription represents 8% from all annual prescriptions, being mostly recommended in over 65 years old patients [1]. Moreover, over-the-counter release of NSAIDs increased their consumption by up to 26% above the recommended release, enhancing the risk of adverse reactions [1].

The study focused on evaluating the possible gastrointestinal adverse effects of one of the most prescribed NSAID, Ibuprofen, and a potential association between nanoparticles and Ibuprofen as a capable method to reduce these side effects. Approximately 1/3 of the patients that take NSAISs develop dyspeptic-like symptoms, epigastric discomfort, associated with nausea, gastroesophageal reflux or early gastric fullness [1]. Although only approximately 10% of NSAIDs chronic users have gastrointestinal symptoms, 70% of them present endoscopical NSAIDs-linked modifications, like mucosal erosions, peptic ulcer or subepithelial hemorrhages [1].

Recently, medical research focused on developing different combinations of nanoparticles and medications, with the scope of finding better treatments, with minimal side effects for multiple pathologies. Regarding nanomedicine and NSAIDs, an inflammation experimental model founded that a 3-week topical administration of methacrylic polymer incapsulated flurbiprofen reduced the gastrointestinal side effects of the drug, regarding oxidative stress parameters and nitric oxide levels [2].

In a study that created a knee osteoarthritis model, the authors developed a special implant formed by polymer nanoparticles conjugated with diclofenac and rodamin, imagistic studies contrast substance, implant that was applied locally. The study concluded that the nanoparticles combined with diclofenac had a prolonged eliberation at synovial level (up to 2 weeks), without enhancing the adverse reacts, compared to the control group (diclofenac only) [3].

Other studies linked NSAIDs' use with ameliorating symptoms and evolution in Alzheimer disease, due to a possible correlation with the disease and chronic cerebral inflammation [4]. Despite this correlation, long-term use of NSAIDs has several side-effects and difficult to impossible penetrability at cerebral level. Research showed that Pegylated polymer nanomolecules approved by the Food and Drug Administration (FDA) and combined with dexibuprofen have been able to cross the hematoencephalic barrier with fewer gastrointestinal side effects than dexibuprofen alone [4]. *In vivo*, dexibuprofen nanomolecules improve spatial memory, diminished the amyloid quantity and the inflammatory response of the glial cells [4].

Superparamagnetic iron oxide nanoparticles were reported recently in a 2026 study to restore gut microbiota, enhance gut barrier function and increased anti-tumor immune cell infiltration in a lung cancer experimental model [5]. Moreover, in the same study, iron oxide nanoparticles seemed to effectively inhibit lung cancer growth at a dose of 12.5 mg/kg [5].

A systematic review published in 2023 revealed the potentially benefic involvement of iron oxide nanoparticles carried by probiotics in iron absorption, in the treatment of iron deficiency anemia [6]. Usually, iron deficiency anemia is treated with dietary iron supplementation, which can be challenging as it can induce mostly gastrointestinal distress, among other issues. The review concluded that probiotics with barrier coatings applied to magnetic nanoparticles can prevent chemical damage in the stomach and increase iron absorption [6].

The objective of this study was the evaluation of oral administration of ibuprofen magnetic nanoparticles – IMNPs, compared with ibuprofen-only treatment regarding possible gastrointestinal side effects. The existence and the degree of severity of gastrointestinal side effects caused by ibuprofen treatment were assessed. In addition, the present study evaluated the possibility of diminishing the gastrointestinal side effects, in cases where ibuprofen is bound to magnetic nanoparticles. The article also studied the existence of significant differences based on the sex variable between ibuprofen-only administration and ibuprofen-loaded nanoparticles administration, correlated to current scientific data.

2. Materials and Methods

2.1. The experimental model used

In elaborating this study, an experimental model was used, which included 30 healthy Wistar white rats, 15 female and 15 males, each being between 16 and 20 weeks of age and weighing between 150 and 350 grams.

The study respected the current ethical guidelines regarding laboratory animals research, having the approval of the Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca (approval no. 2/06.01.2023), as well as the project authorization from the Sanitary Veterinary and Food Safety Directorate Cluj (project authorization no. 265/07.10.2022).

The rats were divided into three equal groups of 10 rats each, 5 female and 5 males. In each group, a daily 10-day treatment via gavage was administered as follows: in the control group (group A), a sodium chloride solution 0.9% (saline solution) was administered, in group B, 7.5 mg/kg/day of simple ibuprofen was administered daily, and group C had daily doses of 7.5 mg/kg/day of IMNPs administered. The 10 days administration of the substances was chosen taking into consideration the usual administration period of anti-inflammatories. From a technical viewpoint, all animals were weighed and marked beforehand and all procedures were done by the same person, in the same conditions and using the same techniques. The rats were kept in optimal conditions, regarding life, nutrition and water ad libitum, following the current ethical guidelines, and they were supervised by specialized personnel, in case of side effects occurrence. The animal evaluation was done prior to the beginning of the experiment (T0) and at the end of the experiment, at day 10 (T1).

Regarding the sex variable, all the evaluated parameters mentioned in the study were assessed both for all the animals in each group and also separately by sex, in order to observe potential differences between males and females.

2.2. Exclusion criteria from the study

The study exclusion criteria included the onset of gastrointestinal side effects caused by the administered substances, namely intractable vomiting, severe digestive hemorrhage, diarrhea or tachycardia, pain of any cause or signs of stress not relieved for a period of time longer than three days, as well as the death or other diseases that occurred independently from the experiment. No rat was excluded throughout the study.

2.3. Preparation method of magnetic nanoparticles functionalized with ibuprofen

First, by using polycondensation reactions, the polylactic acid was synthetized, without using solvents of catalysator. A quantity of 15g of lactic acid was introduced in a Berzelius glass, thermically treated at 140^{0}C for 20 hours. At the end of the chemical process, resulted a solid translucent mass of polylactic acid, which was utilized in the surface absorption of the nanoparticles.

Magnetic nanoparticles' synthesis was based on the co-precipitation standard technique, using $FeCl_2$ (5.94g, 0.03mol), $FeCl_3$ (16.2g, 0.06mol) and distilled water (80ml), in the presence of ammonium hydroxide (200ml) at 80^{0}C under argon. After mixing the solution for 1 hour at 80^{0}C by using a magnetic agitator, the product was cooled at room temperature. Uncovered magnetic nanoparticles (MNP) were separated with an external magnet, washed with water and acetone and dried in an oven at 60^{0}C.

A quantity of 1.2g MNPs was combined with 1.2g of polylactic acid, in a solution based on 20ml of dichloromethane and 20ml ethanol. The mixture was refluxed for 3 hours. Next, the MNPs were isolated and washed with solutions of dichloromethane, ethanol and water, in this order. The nanoparticles were dispersed in a mixture of 20ml ethanol, 50ml distilled water and 0.6g of ibuprofen. After ibuprofen was added,

the mixture was placed at room temperature for 12 hours, under the magnetic agitator. At the end, the magnetic separation of the components was realized. After drying, the resulted nanoparticles were analyzed. Figure 1 shows the process described above.

2.4. Monitored parameters

2.4.1. Evaluation of the administered treatments by the dynamic assessment of the liver toxicity degree: aspartate aminotransferase (AST), alaninaminotransferase (ALT).

The serum parameters of hepatic function (AST, ALT) were dosed from the serum of all the animals of the study, at T0 and T1, using ELISA kits (Sigma Aldrich).

Aspartate aminotransferase (AST, SGOT) is a transaminase-type enzyme, largely distributed in the body, especially in smooth muscles, striated muscles and liver, having various roles, such as aminoacidic metabolism, protein anabolism and catabolism [7,8]. Physiological serum concentrations are within the range of 0-34 U/l, depending on the age, existing renal damage and dosage methods [8]. The normal AST values for Wistar rats are between 50 and 150 U/l, according to scientific literature studies [9]. Plasma AST concentrations that surpass the normal values by a factor of 100 are associated with significant tissue destruction (trauma or acute hypoxia) or with acute, infectious, toxic or ischemic hepatitis [7,8]. Elevated levels of 10-20 times higher than normal are present in the majority of jaundice cases or in myocardial infarction, where growth appears in the first 12 to 36 hours, followed by lowered values in the following 72 hours [8].

Alanine aminotransferase (ALT, SGPT) is a transaminase-type enzyme found in the liver, kidneys, myocardium and skeletal muscles [8]. Normal values are below the threshold of 55 U/l, values that are slightly modifiable depending on the dosage methods [8]. Compared to the AST values, ALT normal values in Wistar rats are estimated to be between 10-40 U/l [9]. Significant level increases in the bloodstream signal extensive liver damage of an infectious nature (viral hepatitis), toxic nature (alcohol, medicine, drugs) or mixed nature, ALT being a more potent marker of hepatocyte disfunction than AST [7,9].

2.4.2. Evaluation of administered treatments by dynamic assessment of oxidative stress parameters

In evaluating the treatment response, side effects or toxic effects due to administration of the treatments, oxidative stress was quantified in the gastric, duodenum and liver tissues, by the dosage of oxidative stress markers. As we consider that the research was extensive, we decided to divide the results into two separate articles. Therefore, in this study we will present the results regarding oxidative stress results at serum and gastric level. Thus, several levels were measured, as follows: reduced glutathione level, oxidized glutathione level and malondialdehyde level (MDA). Glutathione is considered a natural antioxidant, with an important role in the buffering of reactive oxygen species, whose values are correlated with the onset of oxidative stress [10]. On the other hand, in scientific literature, the MDA quantitative increase is linked to the amplification of oxidative stress, through the multiplication of oxygen free radicals [11].

One of the most frequently used methods to show oxidative stress is the dosage of lipid peroxides and aldehydes [12]. The process of lipid peroxidation involves the removal of one hydrogen atom from a polyunsaturated fatty acid molecule [12]. This reaction leads to the formation of lipid radicals in intermediary steps, generating a stable lipid hydroperoxide [12]. Subsequently, the hydroperoxide transforms into an aldehyde, which serves as a stable secondary compound. The stable compounds levels, namely lipid peroxides and aldehydes, can be measured in biological samples [12].

The level evaluation of lipid peroxidation products implies the identification of the colored compound that results from the peroxidation of lipidic structures of various macromolecular substances with 2-thiobarbituric acid, the latter being obtained as a result of the MDA reaction [13]. In order to evaluate lipid peroxidation, the fluorescent method was used and the resulting MDA forms a fluorescent adduct with the thiobarbituric acid [13]. The samples were boiled for one hour, in a 2-thiobarbituric acid 10mM solution, dissolved in a dipotassium phosphate (K_2HPO_4) 75mM solution, with a pH=3 [12]. After rapid sample cooling, the resulting compound was extracted in n-butanol and its concentration was determined in the organic phase, after separation by centrifugation. The emission intensity was measured at a 534nm wavelength, with the help of a Perkin Elmer spectrofluorometer (USA), utilizing synchronous fluorescence with a wavelength difference between excitation and emission ($\Delta\lambda$) of 14 nm [12]. The MDA concentration (nmol/mg protein) was calculated using a calibration curve with known MDA concentrations, that were processed in the same fashion [12].

In determining the GSH and GSSG parameters, a frequently used method was employed [14]. In order to process the tissue homogenate, 50µl were treated with a 450µl 10% m-phosphoric acid solution, followed by centrifugation of 1000 rotations per minute for 10 minutes [14]. To determine GSH, 0.1 ml from the resulting supernatant were used, which were firstly diluted with 1.8 ml of phosphate buffered saline 0.1 M (pH=8), that contained 5nmol/L EDTA [14]. Afterwards, 0.1 ml of o-phthalaldehyde (OPA) were added into methanol (1mg/ml). After a 15-minute incubation time, the fluorescence emitted at 420nm, with a 350nm excitation, was assessed [14]. The GSSG estimation was carried out using 250µl of supernatant, which was incubated for 30 minutes with N-Ethylmaleimide (NEM) 40nmol/L [14]. The following step consisted of adding 0.65ml of NaOH 0,1N, through the same procedure used for GSH, adding however a combination of 0,1ml of NaOH 0.1N and 0.1ml of o-phthalaldehyde solution, instead of phosphate buffered saline. The calculation of GSH and GSSG concentration was based on calibration curves obtained with known GSH and GSSG concentrations, processed in an identical manner [14].

Oxidative stress parameters were dosed in the serum of the animals, at T0 and at T1, at the end of the experiment. At T1, additional oxidative stress parameters were dosed, from tissues harvested from the laboratory animals: gastric, duodenal, hepatic, splenic, renal and colon tissues.

2.5. Statistical data analysis

Data analysis was done using the Microsoft Excel Version 2307 Build 16.0.16626.20028 spreadsheet program, by utilizing the T-test statistical interpretation for independent samples, calculating the average and the standard deviation for each group. In order to ensure that the data follow a normal distribution and can be generalized, we opted for a study containing 30 laboratory animals, randomly divided/randomized in three groups that had different therapies administered. The differences were regarded as statistically significant if $p<0.05$.

3. Results

3.1. Ibuprofen-functionalized magnetic nanoparticles analysis

Infrared absorption spectrum was assessed between 400-4000 cm^{-1} with a JASCO FTIR-6100 spectrophotometer, on a pressed pellet formed by nanoparticles covered with potassium bromide (Figure 2). Fourier-transformed infrared spectroscopy (FTIR) a evaluated the presence of the cover of the MNPs. A high absorption at the 540 cm^{-1} wavelengths, observed both in the MNPs and IMNPs spectroscopy, was attributed to the presence of iron. The C=O bundle wavelength at 1750 cm^{-1} was observed only for IMNPs. The wave band at 1090 cm^{-1} was associated with carbon-hydrogen bundle, correlated with the benzenic nucleus deformation.

Transmission electronic microscopy images of IMNPs showed the presence of polylactic acid and ibuprofen coating at the nucleus level of the IMNPs (Figure 3). The INMPs had a spherical shape and a diameter between 14 and 17 nm, values that were inferior to the standard dimensions of the magnetite, thus the superparamagnetic properties of the IMNPs.

3.2. *Evaluation of administered treatments by dynamic appreciation of liver toxicity degree: aspartate aminotransferase (AST), alanine aminotransferase (ALT)*

A comparative analysis of serum AST levels between study groups at T1 can be found in Figure 4 (a). The average of the witness group (68.66±8.73) showed decreased values compared to the average of the group treated with ibuprofen (99.004±24.13) and compared to the average of the group treated with IMNPs (85.07±14.34), with statistically significant differences ($p<0.05$). The groups which had ibuprofen and IMNPs administered presented a higher degree of liver damage than the witness group. There were no statistically significant differences between the group treated with ibuprofen and the one treated with IMNPs, but increased AST values were observed in the group treated with ibuprofen, compared to the IMNPs-treated group.

Figure 4 (b) shows a comparative analysis between study groups serum ALT levels at T1. The average in the ibuprofen-treated group (94.08±17.12) and in the IMNPs group (75.23±10.28) showed increased ALT values compared to the witness group (68.51±7.18), revealing a higher degree of liver damage than the witness group. There were statistically significant differences between the witness group and the ibuprofen-treated group ($p<0.05$), while between the witness group and the IMNPs group, statistically significant differences were not found. The IMNPs-treated group showed an ALT average lower than the ibuprofen group and the differences were found to be statistically-significant ($p<0.05$).

The mean value of ASAT was lower in female subjects compared to male subjects regarding the groups that received ibuprofen or IMNPs. For the control group, the differences between sexes were minor, with a slight increase of ASAT values for males, with no statistical signification (Figure 5 (a)).

The comparison of ASAT values regarding sex variable is illustrated in Figure 5 (b). There were statistically significant differences for the IMNPs group, where the ASAT values were higher in males compared to females. For the ibuprofen group, there were also higher values for the male rats, but without statistical differences compared to females. For the control group, the female rats presented increased values compared to the male rats, but with no statistical signification.

3.3. Evaluation of administered treatments by dynamic appreciation of oxidative stress parameters

3.3.1. Oxidative stress treatment in relation to malondialdehyde levels at serum and gastric level

The comparative analysis of serum MDA levels between the study groups at T1 is shown in Figure 6 (a). The average of the group treated with ibuprofen (2.96±0.81) and the average of the group treated with IMNPs (3.47±0.53) showed lower MDA values compared to the witness group average (3.39±0.82). MDA values dosed in the ibuprofen-treated group were the lowest, compared to the witness group and the IMNPs group, thus the oxidative stress levels were the lowest in the ibuprofen-treated group. The IMNPs group revealed an increased MDA average in comparison to the witness group, the increase being visible in comparison to the ibuprofen-treated group, as well. The $p>0.05$ value did not reveal statistically-significant differences.

Figure 7 (a) shows the distribution of average sex values of serum MDA levels in the witness group, in the ibuprofen group and in the IMNPs group. For the group

treated with ibuprofen, statistically significant differences between the male and female subjects were observed, with increased MDA values appearing in male subjects. Female subjects presented higher MDA values than male subjects, when comparing the witness group and the IMNPs group, but the differences were not statistically significant.

The comparative analysis of gastric MDA values between the study groups at T1 is illustrated in Figure 6 (b). The witness group average (0.10±0.04) presented the lowest gastric MDA values, in correlation with the ibuprofen-treated group average (0.16±0.05) or with the IMNPs-treated group average (0.18±0.06), with statistically significant differences in both cases ($p<0.05$). Comparing the ibuprofen group and the IMNPs group, it was revealed that the ibuprofen group showed inferior MDA values, with an increase in oxidative stress in the IMNPs group, in the absence of statistical significance.

Figure 7 (b) shows the sex distribution of the average gastric MDA values in the groups at T1. There were no statistically significant differences observed, but the graph shows an increase in average MDA values in female subjects compared to male subjects in all the groups.

3.3.2. The evaluation of oxidative stress related to reduced glutathione levels at serum and gastric levels

Figure 8 (a) represents a comparative analysis of serum GSH (reduced glutathione) levels between the study groups at T1. In the three groups, progressively increased average values of serum GSH were found, in the witness group (4.24±1.74), in the ibuprofen-treated group (7.65±2.71) and in the IMNPs-treated group (8.93±1.13). Statistically significant differences were detected between the witness group and the ibuprofen-treated group, and between the witness group and the IMNPs-treated group, respectively ($p<0.05$). In evaluating the ibuprofen-treated group, the findings showed that the ibuprofen-treated group had inferior values to the IMNPs-treated group, but the differences were not statistically-significant.

Figure 9 (a) illustrates the sex distribution of average serum GSH values for the study groups, with the appearance of increased averages in male subjects, compared to female subjects. No statistically significant differences between the variables were found in either of the examined groups.

A comparative analysis between the study groups' gastric GSH levels at T1 is presented in Figure 8 (b). The witness group average (5.09±0.79) was the highest, in comparison with the ibuprofen group average (4.15±1.32) and with the IMNPs group average (3.31±0.69), showing statistically significant differences between the witness group and the ibuprofen group and between the witness group and the IMNPs group, respectively ($p<0.05$). In the analysis of differences between the ibuprofen group and the IMNPs group, a decrease in the value of the IMNPs group was observed, thus a smaller level of oxidative stress, with no statistically significant differences, however.

Sex distribution of gastric GSH average values appears in Figure 9 (b). Each study group presents higher average values in male subjects than in female subjects. The differences were not statistically significant in either of the three groups.

3.3.3. Oxidative stress evaluation in correlation to oxidated glutathione levels at serum and gastric level

A comparative analysis of serum GSSG (oxidated glutathione) levels between the study group at T1 is illustrated in Figure 10 (a). In reporting the differences between the witness group (1.80±0.71) and the ibuprofen group (1.45±0.24), a decrease in the values of the ibuprofen group was revealed, a finding applicable when comparing the witness group (1.80±0.71) and the IMNPs group (1.63±0.22), as well, with lower serum GSSG values in the latter group. The ibuprofen group presented inferior

values compared to the IMNPs group, with no statistically significant differences based on serum GSSG levels.

Figure 11 (a) shows the sex distribution of the average serum GSSG levels in the study groups. Thus, it was observed that female subjects showed increased averages of serum GSSG than the female subjects, when the ibuprofen group and the IMNPs group were analyzed. However, the reverse was shown to happen in the witness group, which presented higher serum GSSG values in male subjects. In the aforementioned situations, no statistically significant differences were revealed.

Figure 10 (b) illustrates a comparative analysis of gastric tissue GSSG levels between the study groups at T1. Statistically significant differences were observed between the witness group (0.13±0.07) and the ibuprofen group (0.24±0.15), with an increase in GSSG values in the ibuprofen group ($p<0.05$). The same conclusions were drawn when comparing the witness group (0.13±0.07) and the IMNPs group (0.17±0.05), the latter showing increased GSSG values, in the absence of statistically significant differences. No statistically significant differences were found between the ibuprofen group (0.24±0.15) and the IMNPs group (0.17±0.05). However, decreased GSSG values were revealed in the IMNPs-treated group.

Sex distribution regarding GSSG gastric levels shows a superior mean value for female in the control and ibuprofen group, but little differences between sexes for the IMNPs group, with overall no statistical differences between groups (Figure 11 (b)).

3.3.4. GSH/GSSG ratio at serum and gastric levels for the evaluation of oxidative stress

Figure 12 (a) presents a comparative analysis of serum GSH/GSSH levels between the study groups at T1. The witness group average (2.69±1.52) presented inferior values than the ibuprofen-treated group (5.49±2.41) and then the IMNPs-treated group (5.52±1.24), respectively, with the average of the IMNPs group (5.52±1.24), with statistically significant differences between the witness group and the other two groups ($p<0.05$). The IMNPs group's average values were increased (5.52±1.24), compared to the ibuprofen group average values (5.49±2.41), with no statistically significant differences between the two groups.

Figure 13 (a) illustrates the sex distribution of serum GSH/GSSG values between the study groups at T1, revealing higher values in male subjects than in female subjects, in all three study groups, with no statistically significant differences between them.

Figure 12 (b) presents a comparative analysis of gastric tissue GSH/GSSG levels between the study groups at T1. Lowered values of GSH/GSSG levels were observed both in the ibuprofen group (22.00±11.53) and in the IMNPs group (20.10±7.11), compared to the witness group (48.58±23.79), with statistically significant differences appearing between the witness group and the ibuprofen group and between the witness group and the IMNPs group, respectively ($p<0.05$). The IMNPs group registered inferior values (20.10±7.11) than the ibuprofen group (22.00±11.53), without statistically significant differences.

Figure 13 (b) illustrates the sex distribution of average GSH/GSSG values in the gastric tissue, showing superior values in female subjects than in male subjects in all study groups, with no statistically significant differences.

3.2. Figures, Tables and Schemes

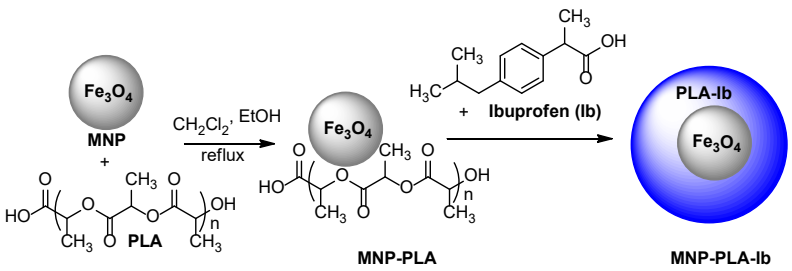


Figure 1. Ibuprofen-functionalized iron oxide magnetic nanoparticles synthetis reaction (MNP-magnetic nanoparticles, PLA-polylactic acid, MNP-PLA-Ib-magnetic nanoparticles covered with polylactic acid and ibuprofen)

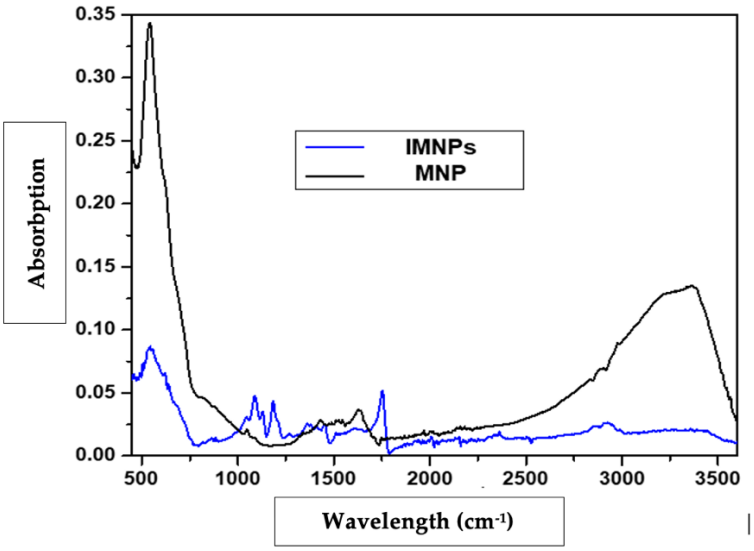


Figure 2. FTIR analysis of MNPs and IMNPs

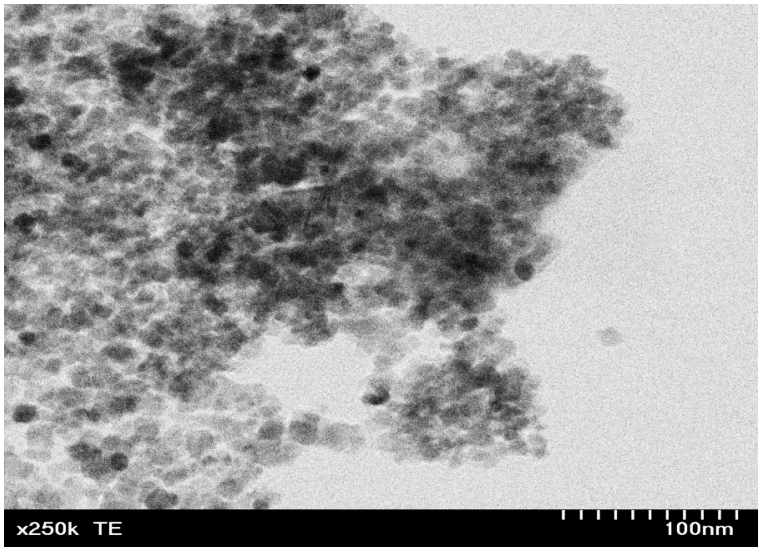
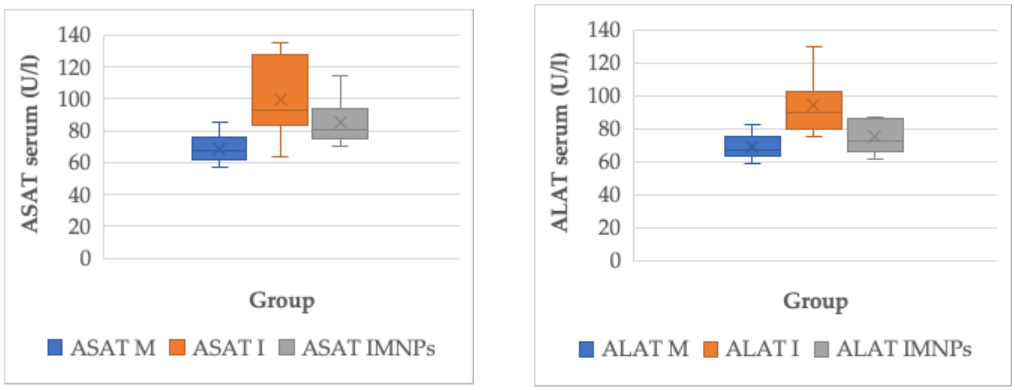
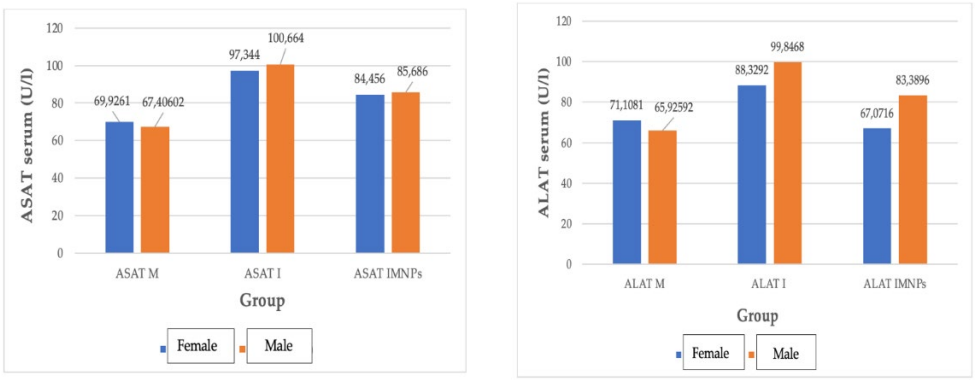


Figure 3. Transmission electron microscopy image of IMNPs



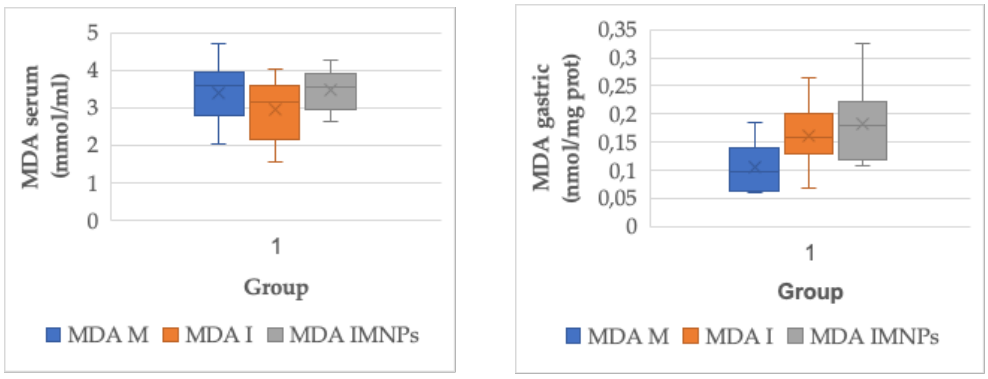
(**a**) (**b**)

Figure 4. Comparative analysis of serum ASAT (**a**) and ALAT (**b**) levels between study groups at T1



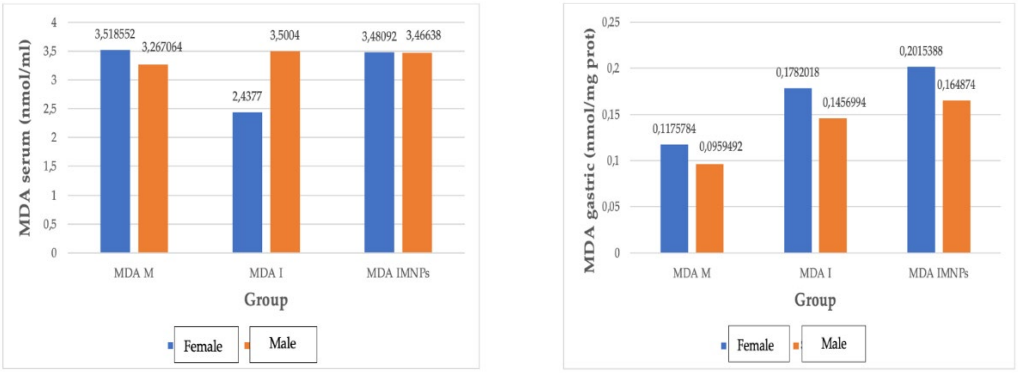
(**a**) (**b**)

Figure 5. Sex distribution of serum ASAT (**a**) and ALAT (**b**) levels between study groups at T1



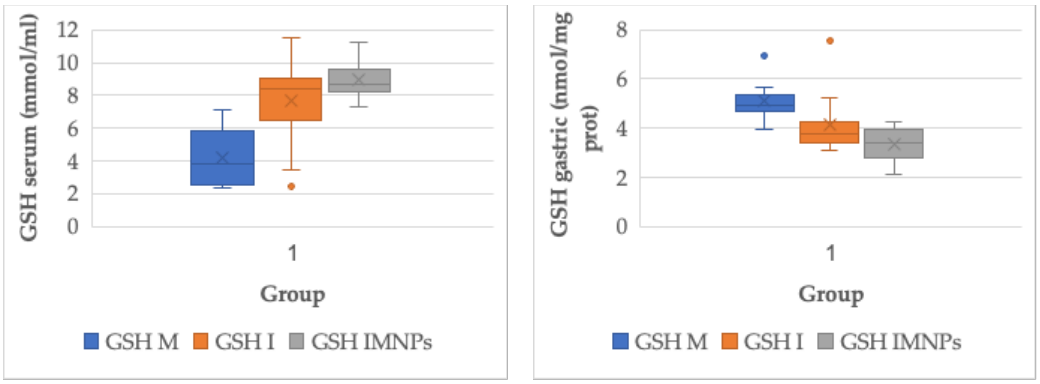
(**a**) (**b**)

Figure 6. Comparative analysis of MDA serum (**a**) and gastric (**b**) levels between study groups at T1



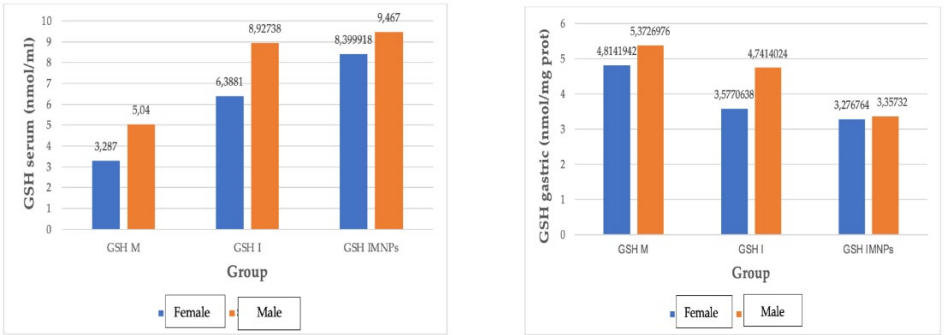
(a) **(b)**

Figure 7. Sex distribution of MDA serum (**a**) and gastric (**b**) levels between study groups at T1



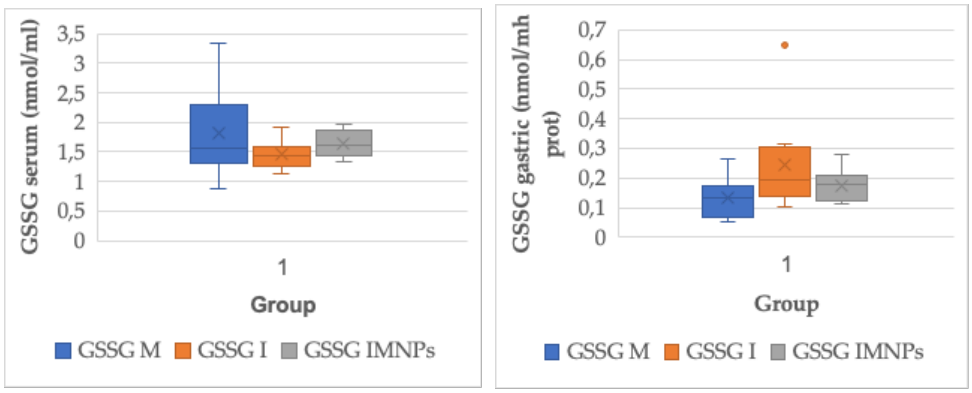
(**a**) (**b**)

Figure 8. Comparative analysis of GSH serum (**a**) and gastric (**b**) levels between study groups at T1



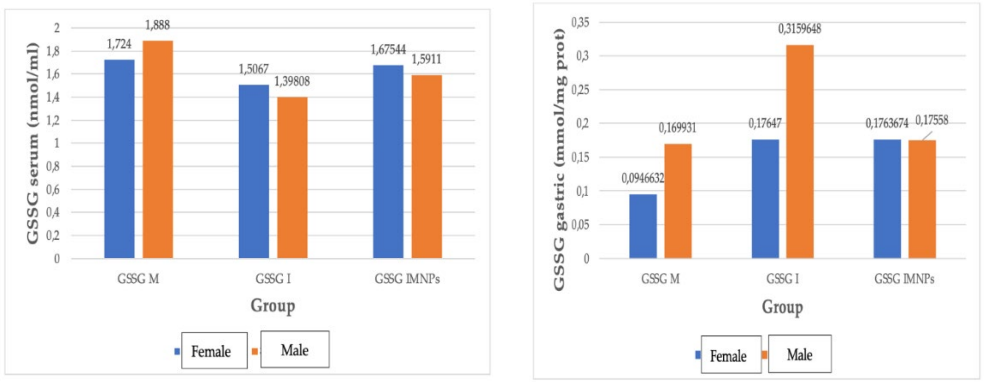
(**a**) (**b**)

Figure 9. Sex distribution of GSH serum (**a**) and gastric (**b**) levels between study groups at T1



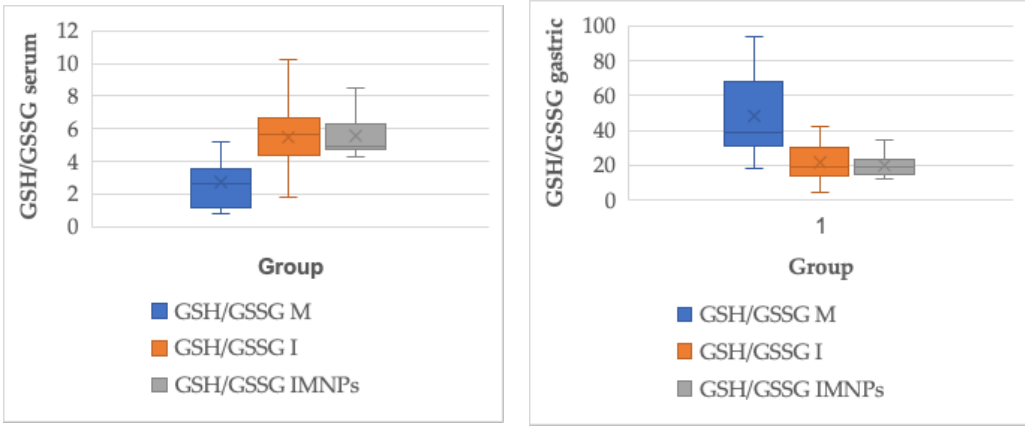
(a) **(b)**

Figure 10. Comparative analysis of GSSG serum (**a**) and gastric (**b**) levels between study groups at T1



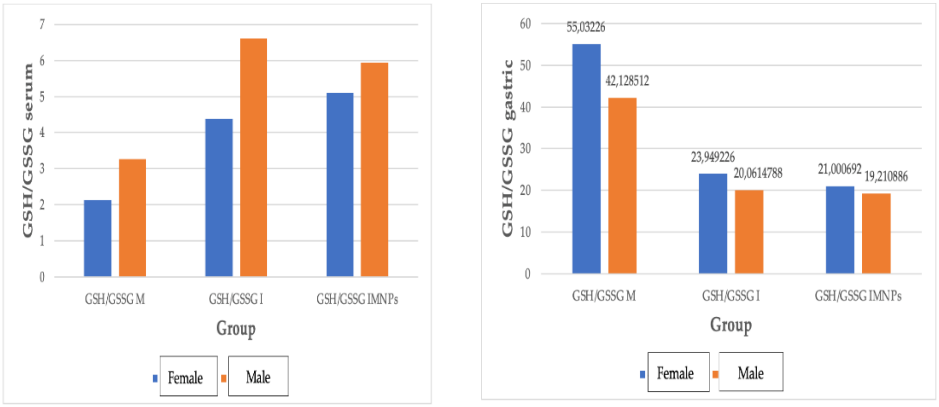
(a) **(b)**

Figure 11. Sex distribution of GSSG serum (**a**) and gastric (**b**) levels between study groups at T1



(**a**) (**b**)

Figure 12. Comparative analysis of GSH/GSSG serum (**a**) and gastric (**b**) levels between study groups at T1



(**a**) (**b**)

Figure 13. Sex distribution of GSSG serum (**a**) and gastric (**b**) levels between study groups at T1

4. Discussion

The use of iron oxide magnetic nanoparticles as drug transport mechanism seems to represent a viable way to diminish the adverse effects and to enhance the therapeutic effect [15]. Campos I et al. studied the cytotoxic and genotoxic effect of ibuprofen functionalized iron oxide magnetic nanoparticles, showing a reduced toxicity for the nanoparticles and their potential as biomedical delivery systems [16].

The originality of the present study consists in the unique approach of the research theme, regarding the use of ibuprofen functionalized magnetic nanoparticles in NSAID-induced gastrointestinal side effects. At the moment of study conception, the authors couldn't find any other similar studies. Dash S et al researched the use of magnetic nanoparticles in prostate cancer, pancreatic cancer, glioma and myocardial infarct, directing the nanoparticles by using external imaging control (MRI) [17]. Unlike the cited article, our research did not use any external guidance of the nanoparticles, as we wanted to observe their unaffected behavior at digestive tract level.

The present study observed an antioxidant response of the IMNPs compared to the control group or ibuprofen alone, with statistically significant differences regarding GSH and GSH/GSSG levels. Also, for the IMNPs group, increased levels of MDA and decreased values for GSSG were observed, by comparison with the control group. On the other hand, IMNPs group presented a higher oxidative stress when compared to the ibuprofen group, but without clinical significance, and the results need supplementary analysis. Similar to the present research, in a study elaborated by Romano M et al it has showed that ibuprofen MNPs reduced oxidative stress by lowering the nitric oxide levels compared with ibuprofen alone [18]. On the other hand, IMNPs appear to have a lower hepatic toxicity, as they reduced the ASAT and ALAT values, compared with the control and ibuprofen group, results that are similar with other reported findings, like research published in 2024, that found significantly decreased aspartate aminotransferase, lactate dehydrogenase and portal pressure in cirrhotic rats that received nitric oxide nanoparticles compared with nanoparticles alone [19].

Regarding de sex variable, the data suggests a higher inflammatory response for male subjects, with increased values for hepatic transaminases and oxidative stress parameters. These results are similar with other reported values from the literature, suggesting a lower inflammatory response for female subjects, which appears to be modulated by hormonal and genetic influences [20].

The study's limitations may consist in the relative low number of animals that were used, but also in the short amount of time in which the NSAIDs and IMNPs effects were observed. NSAIDs side effects are usually developed after chronic use, depending on several co-factors, but, on the other hand, we wanted to limit the amount of time during which the animals were exposed to possible harms and we

also took into consideration the usual NSAIDs treatment period, respectively 10 days. We appreciate that more studies are needed in order to confirm our findings but also to follow up the effects of a longer treatment period.

5. Conclusions

The study showed that ibuprofen functionalized iron oxide magnetic nanoparticles appear to reduce the inflammatory response induced by NSAIDs administration at serum and gastric level, representing a step forward in developing better pain treatments, with fewer to no side effects. However, more studies are needed in order to formulate a conclusion regarding the matter and for a better understanding of the behavior of nanoparticles.

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Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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