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Research Article

Immunomodulatory and hematological effects induced by diclofenac, ibuprofen or paracetamol toxicity in Swiss albino mice

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ABSTRACT

Anti-inflammatory drugs (both COX-2 inhibitors and nonselective non-steroidal anti-inflammatory drugs = NSAIDs), paracetamol and opioid agents are associated with potentially different adverse events with varying degrees of efficacy. The present work was conducted to elucidate the haemato-immunological changes in mice when treated with diclofenac (Diclo), ibuprofen (Ibu) and paracetamol (Para). Mice were intraperitoneally administered with Diclo (7.4 mg/kg and 14.8 mg/kg), Ibu (60 mg/kg and 120 mg/kg) or Para (36.7 mg/kg and 73.4 mg/kg) daily for one month against saline-treated mice served as control. Diclo administration (14.8 mg/kg) caused decrease in RBCs count, Hb content and Hct%, depending on dose toxicity, while paracetamol and ibuprofen treatment showed increase in RBCs count, Hb content and Hct%. Additionally, all tested drugs induced activities of IgM and C-reactive protein in serum and caused perturbations in absolute and relative weight of immune related organs. Further, Diclo and Para treatments reduced levels of IgG in dose dependent manner however, Ibu administration enhanced activities of IgG that was reduced with increasing dose of Ibu. And activities of serum complement component C3 was diminished after administration of tested drugs activating alternative complement pathway. The implication of this research is that long use of diclofenac, ibuprofen or paracetamol may cause immunotoxic and hematotoxic effects in mice; and the dose plus the duration of treatment may augment their toxicity probably due to immune modulatory effects. Further studies are needed to assess the relevance between Diclo, Ibu or Para treatment and immunological and hematological perturbations.

Keywords: Immunological and hematological studies; Diclofenac; Ibuprofen; Paracetamol; Toxicity.

1. INTRODUCTION

The main analgesic agents that generally used for the most popular types of pain, include non-steroidal anti-inflammatory drugs (NSAIDs; both traditional non-selective and cyclo-oxygenase (COX)-2 selective agents), paracetamol and opioids [1]. NSAIDs have exhibited excellent efficacy in the control of acute pain producing both anti-inflammatory and analgesic effects [2, 3] by inhibition of prostaglandin synthesis via the COX enzyme that regulates normal physiological turnover of prostaglandin and maintains integrity of gastric

lining and renal homeostasis [4]. The therapeutic anti-inflammatory action of NSAIDs is produced by the inhibition of COX-2, while the potential damaging pernicious effects emerge from inhibition of physiological COX-1 activity [5].

Traditional NSAIDs such as ibuprofen or diclofenac, have been established to inhibit COX enzyme activity [6] resulting in the prevention of synthesis of prostaglandins that interfere pivotal physiological functions, including gastric cytoprotection, maintenance of renal blood flow, and platelet activation [7]. Ibuprofen (Ibu), an over-the-counter (OTC) drug, is one of the most widespread used NSAIDs as an analgesic, antipyretic, and anti-inflammatory drug globally [8, 9]. Although, NSAIDs are commonly considered to have high safety profiles, the frequent and general use of ibuprofen and other NSAIDs is likely to increase the prevalence of their adverse effects. Ibuprofen and other NSAIDs are commonly linked to gastrointestinal (GI) toxicity [10, 11] and alternation in renal function [12, 13]. So, regular toxicological evaluation of NSAIDs becomes essential.

Diclofenac (Diclo) is a widely circulated drug, used in humans and animals for the treatment and management of inflammation, fever and pain associated with disease or injury of domestic livestock and humans, regarding to its anti-inflammatory, analgesic and antipyretic properties, however it has severe pathologic conditions such as peptic ulceration, gastrointestinal bleeding, hepatotoxicity, renal papillary necrosis and renal failure on long-term of the drug administration [14-16]. Anti-inflammatory, antipyretic and analgesic action of Diclo is related to inhibition of prostaglandin synthesis from arachidonic acid by inhibition of cyclooxygenase (COX) [17]. Diclofenac was found to cause pathological changes in kidneys of the vultures leading eventually to the gout [18] and a rare but potentially fetal hepatotoxicity that may be related to reactive metabolites formation [19-21].

Alternative to NSAIDs, paracetamol (Para) is one of the most popular drugs around the world, available without a prescription, especially in childhood [22, 23]. Similar to NSAIDs, Para has a potent antipyretic and analgesic actions but without anti-inflammatory activity and a weak inhibition of prostaglandins synthesis [24, 25]. Also, it has a spectrum of action analogous to that of NSAIDs and mostly resembles the COX-2 selective inhibitors. In spite of its wide use, the mechanism of action of acetaminophen has not been fully elucidated, but it is commonly agreed that it inhibits COX-1 and COX-2 through metabolism by the peroxidase function of these isoenzymes, the possibility exists that it inhibits a so far unidentified form of COX, perhaps COX-3 [26] or there is another mechanism of action that include the effects of both the peripheral (inhibition of COX activity) and central (COX, descending serotoninergic pathways, L-arginine/NO pathway, cannabinoid system) antinociceptive processes as well as the redox mechanism [27] concluding that Para has a multifactorial mechanism of action, which may include the activation of different pain pathways hence the difficulty in clarifying the delicate mechanism of action [28]. Although Para is safe and well tolerated when taken in the usual therapeutic dose, its overdose is fairly common and often linked with hepatic and renal damage in both humans and experimental animals [29, 30].

Due to dearth of information from literature on the adverse effects of Diclofenac sodium, Ibuprofen and Paracetamol on immunological and hematological parameters in mice, therefore the present study was planned with the objective to investigate the immunomodulatory and hematological effects of repeated doses of diclofenac, ibuprofen or paracetamol in mice for one month.

2. MATERIALS AND METHODS

2.1. Experimental animals

Male Swiss albino mice were obtained from Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt. Animals were 4-6 weeks old and weighed between 20-28 g at the beginning of the experiment. They were handled and kept in a specific pathogen-free facility at Faculty of Science, Tanta University in accordance with the ethical guidelines of Egyptian National Research Center, Cairo, Egypt and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All animals were housed under the same environmental conditions for 1 week before experimentation for acclimatization and to

ensure normal growth and behavior. The mice were housed under standard laboratory conditions (temperature 22°C ± 2°C; 12 h light-dark cycle) and kept in plastic cages with free access to the commercial basal food and water.

2.2. Drugs

The tested drugs: diclofenac sodium (Diclo) (each tablet contains 50 mg diclofenac sodium, Novartis Pharma, Cairo, Egypt), ibuprofen (Ibu) (each tablet contains 200 mg ibuprofen, Kahira Pharaceuticals & Chemical Industries Company, Cairo, Egypt), and paracetamol (Para) (each tablet contains 500 mg of active drug, Arab Drug Company, Cairo, Egypt) were purchased from public drug store (Tanta, Egypt). Each tablet was crushed to fine powder and dissolved in saline at appropriate concentrations.

2.3. Experimental design

Mice were divided into seven groups of ten animals each. Group1 was administrated saline (i.p.) as a control group, group 2 and group 3, i.p. inoculated with Diclo (14.8 mg/kg, 5 time less than LD50 and 7.4 mg/kg, 10 time less than LD50 respectively [31], group 4 and group 5, i.p. injected with Ibu (120 mg/kg, 5 time less than LD50 and 60 mg/kg, 10 time less than LD50 correspondingly [32], group 6 and group 7, i.p. injected with Para (73.4 mg/kg, 5 time less than LD50 and 36.7 mg/kg, 10 time less than LD50 separately) [33] daily for a period of one month. At the end of treatment, three mice from each group were euthanized by cervical dislocation at fasting state. Preceding to the scarifying, blood samples were collected from retro-orbital plexus for immunological and hematological analyses.

2.4. Hematological analysis

Blood parameters were proceeded for hematological analysis using a Nihon Kohden automated hematology analyzer (model MEK-6318K, Japan), including red blood cell count (RBC) (10^6/µl), hemoglobin concentrations (Hb g/dl), hematocrit percentage (Hct%) and platelet count (Plt) (10^3/µl).

2.5. Preparation of sera samples

At the end of experiment, three mice from each group were euthanized by cervical dislocation at fasting state. Prior to a euthanizing, blood samples were collected from retro-orbital plexus in plastic test tubes and allowed to stand for 3 h to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and the clear sera samples were aspirated off and stored frozen at -80°C for immunological analyses. Evaluation of the different immunological parameters: Complement component C3, C4 and C-reactive protein (CRP). Serum levels of IgG and IgM in exposed mice were performed using enzyme linked immuno-sorbent assay (ELISA) as described by [34, 35] in triplicate for each sample. The manufacturer's instructions for each parameter were strictly followed in the course of the investigations.

2.6. Body weight gain and immune-related organs relative weight

Just before killing after 30 days, final body weight of mice in all experimental groups was recorded. Upon being killed, the spleen, lymph nodes and thymus were removed aseptically, weighed and their relative organ weights (ROW) were calculated according to Aniagu et al. [36] using the following formula:

ROW = [Absolute organ weight (g) / body weight of mice on sacrifice day (g)] × 100.

Percentage weight gains of mice (WG%) were calculated according to Tukmechi et al. [37] using the following formula: WG% = (final body weight - initial body weight) × 100/initial body weight).

2.7. Statistical analysis

The data were expressed as mean ± standard error of the mean (n = 3). Statistical comparisons among prospective groups were analyzed using a one-way analysis of variance (ANOVA) as part of an SPSS software package (v.16.0 for Windows, 2007; SPSS, Inc., Chicago, IL). Statistical significance was determined by a post hoc test followed by Dunnett's multiple comparison tests to compare treatment means versus respective controls. Significant differences are indicated as follows:

*, P < 0.05; **, P < 0.01 for significant and highly significant differences, respectively.

3. RESULTS

The current study was conducted to investigate the adverse effects of daily administration of different pain killers like Diclofenac sodium (Diclo), Ibuprofen (Ibu) and Paracetamol (Para) intraperitoneally for one month on immunological and hematological changes in mice. The present findings indicated that 1-month continuous treatment with Diclo, Ibu and Para has altered the immuno-hematological parameters in the processed mice.

The adverse effects of Diclo, Ibu and Para administration on body weight gain, absolute and relative weight of liver and kidney in the albino mice are indicated in Table 1. The obtained data revealed non-significant decrease in the monitored weight gain in all treatments used in this study, when compared to saline treated group. Further, absolute and relative weight of liver and kidney showed non-significant changes comparing to saline-treated mice.

Table 1. Changes in body weight, percentage of body weight gain, absolute and relative weight of liver and kidney after treatment with Diclo, Ibu or Para.

Treatment	Body wt			Liver		Kidney	
	Initial Body WT	Final Body WT	Body WT gain (%)	Absolute WT	Relative WT	Absolute WT	Relative WT
Control	23.47±2.39	30.11±3.12	28.29±3.79	1.68±0.17	5.63±0.58	0.5±0.06	1.64±0.05
Diclo (7.4 mg/kg)	22.00±0.61	28.47±0.81	29.44±2.61	1.89±0.16	6.33±0.26	0.45±0.02	1.51±0.01
Diclo (14 mg/kg)	20.80±0.12	25.40±0.81	22.10±3.58	1.42±0.10	5.92±0.18	0.38±0.01	1.59±0.15
Ibu (60 mg/kg)	23.23±0.66	28.37±0.55	22.21±2.75	1.40±0.08	5.36±0.21	0.45±0.05	1.73±0.14
Ibu (120 mg/kg)	24.33±1.79	29.23±0.75	21.01±5.93	1.57±0.14	5.35±0.34	0.4±0.02	1.36±0.03
Para (36.7 mg/kg)	23.80±0.81	29.51±0.7	24.42±6.73	1.71±0.08	5.79±0.24	0.41±0.01	1.40±0.02
Para (73.4 mg/kg)	24.47±2.09	30.67±2.37	26.07±7.71	1.61±0.21	5.22±0.44	0.46±0.03	1.49±0.02

Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

Table 2. Changes in absolute and relative weight of spleen, thymus and lymph node of mice after treatment with Diclo, Ibu or Para.

Treatment	Spleen		Thymus		Lymph node	
	Absolute WT	Relative WT	Absolute WT	Relative WT	Absolute WT	Relative WT
Control	0.22±0.04	0.72±0.06	0.05±0.01	0.17±0.02	0.08±0.003	0.26±0.02
Diclo (7.4 mg/kg)	0.27±0.05	0.89±0.14	0.07±0.01	0.25±0.03	0.08±0.003	0.28±0.01
Diclo (14 mg/kg)	0.28±0.05	1.15±0.14**	0.04±0.01	0.17±0.03	0.06±0.006	0.25±0.02
Ibu (60 mg/kg)	0.14±0.02*	0.53±0.09*	0.04±0.01	0.15±0.02	0.06±0.010	0.24±0.04
Ibu (120 mg/kg)	0.13±0.02**	0.43±0.06**	0.04±0.00	0.15±0.01	0.05±0.006	0.17±0.02
Para (36.7 mg/kg)	0.17±0.01	0.57±0.03	0.03±0.00	0.11±0.01	0.04±0.006*	0.14±0.02*
Para (73.4 mg/kg)	0.16±0.05*	0.51±0.13*	0.07±0.02	0.22±0.06	0.05±0.010*	0.15±0.04*

Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

The relative and absolute weights of immune organs (spleen, thymus and lymph node) in albino mice are illustrated in Table 2.

The present finding showed that Diclo (7.4 mg/kg and 14.8 mg/kg) treatments resulted in significant increase in spleen relative weight, whereas there was significant decrease with Ibu (60 mg/kg and 120 mg/kg) and Para (73.4 mg/kg) administration and non-significant decrease with Para (36.7 mg/kg) treatment compared to saline-treated mice. Moreover, Diclo (7.4 mg/kg and 14.8 mg/kg) and Ibu (60 mg/kg and 120 mg/kg) treatments reduced the relative weight of lymph node in dose dependent manner, and Para (36.7 mg/kg and 73.4 mg/kg) treated mice showed significant decrease relative to saline-treated mice. There were no significant differences in the relative and absolute weight of thymus in all treated mice when compared with saline-treated mice.

During the present study, the effects of Diclo, Ibu and Para on RBC and Plt in the Swiss albino mice are shown in figure 1. The results indicated that there is slight increase in RBCs count with Diclo (7.4 mg/kg), Ibu (60 mg/kg and 120 mg/kg) - treated mice and a significant increase with administration of Para (73.4 mg/kg), but no change in RBCs with Para (36.7 mg/kg) against control (Fig. 1-A). In the term of Plt, Ibu (60 mg/kg), Para-treated (36.7 mg/kg and 73.4 mg/kg) mice did not differ significantly from the saline-treated group, however Diclo-inoculated (7.4 mg/kg and 14.8 mg/kg) mice had increased Plt count and Ibu-treated (120 mg/kg) mice showed the highest significant value comparing to control mice (Fig. 1-B).

The results further revealed that significant increase in Hb content with high dose of Para and Ibu-injected mice as compared to saline-treated mice; while low dose of Diclo, Para, Ibu-treated mice indicated minor increase in Hb content. Also, there was slim decrease in Hb content of high dose of Diclo-inoculated mice (Fig. 2-A). Moreover, HCT percentage (Fig. 2-B) displayed a small elevation in Diclo (7.4 mg/kg); Ibu (60 mg/kg and 120 mg/kg) and Para-treated (36.7 mg/kg and 73.4 mg/kg) mice when compared to saline-treated group, even though Diclo-treated (14 mg/kg) mice presented minor decrease in HCT%.

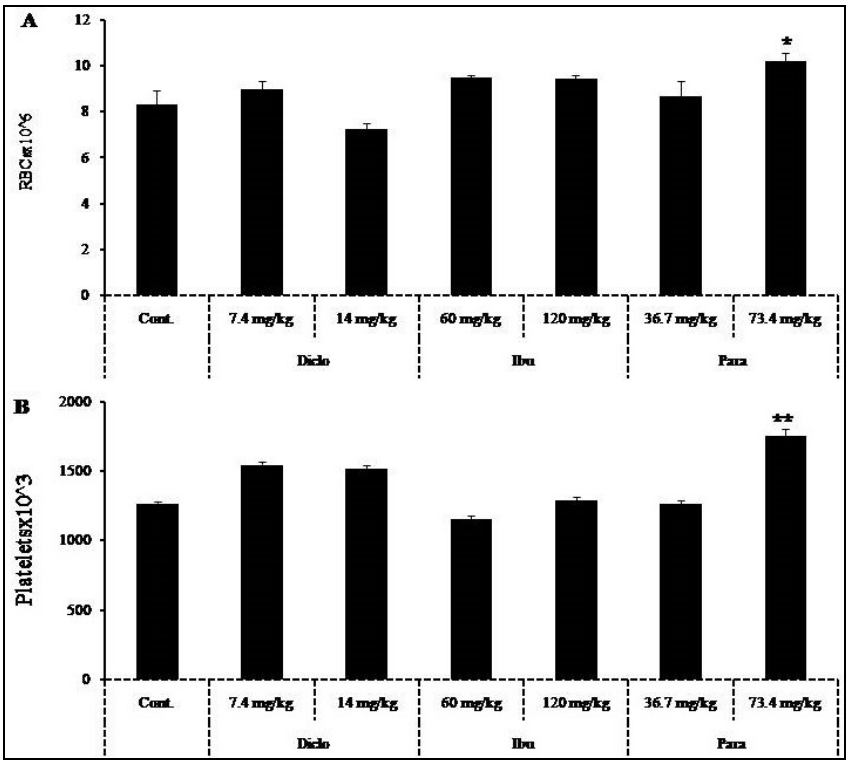

Figure 1. Effect of repeated administration of Diclo, Ibu or Para on RBCs and platelets count. Mice treated with saline (control), Diclo (7.4 mg/kg, 14.8 mg/kg), Ibu (60 mg/kg, 120 mg/kg), Para (36.7 mg/kg, 73.4 mg/kg) intraperitoneally (i.p.) daily for one month. Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

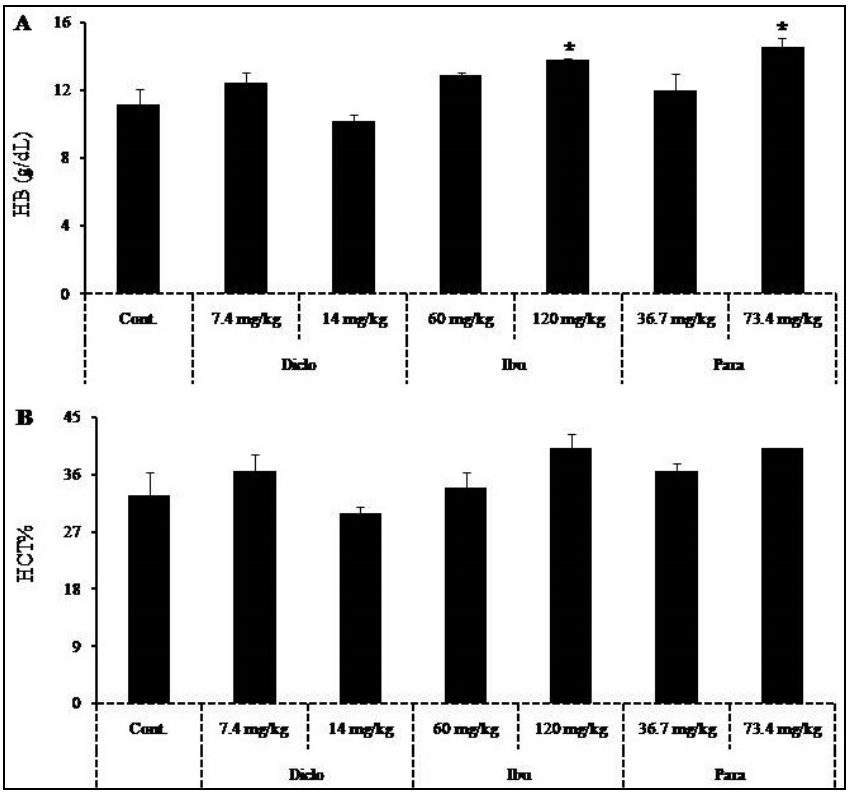

Figure 2. Changes in HB concentration and HCT% after administration of Diclo, Ibu or Para. Mice treated with saline (control), Diclo (7.4 mg/kg, 14.8 mg/kg), Ibu (60 mg/kg, 120 mg/kg), Para (36.7 mg/kg, 73.4 mg/kg) intraperitoneally (i.p.) daily for one month. Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

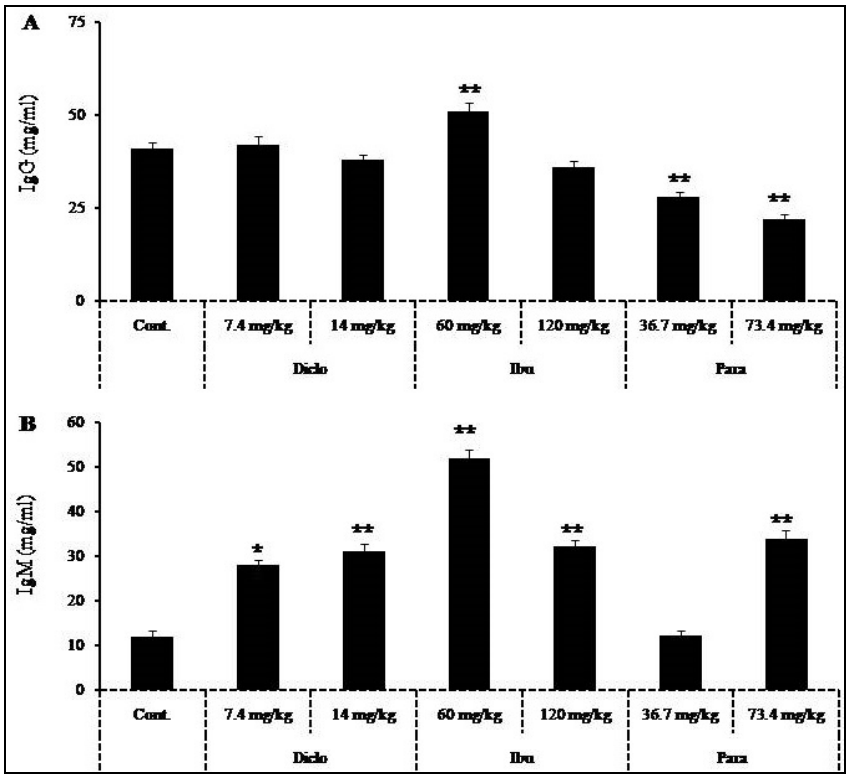

Figure 3. Changes in IgG and IgM concentration in PB after repeated administration of different pain killers. Mice treated with saline (control), Diclo 1 (7.4 mg/kg), Diclo 2 (14.8 mg/kg), Para 1 (36.7 mg/kg), Para 2 (73.4 mg/kg), Ibu 1 (60 mg/kg), Ibu 2 (120 mg/kg) intraperitoneally (i.p.) daily for one month. Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

In figure 3-A, all treatments had reduced IgG concentration in dose dependent manner. Diclo treatment decreased IgG concentration from 42 to 38 (mg/ml), Para treatment from 28 to 22 (mg/ml), and Ibu treatment from 51 to 36 (mg/ml) with increasing the dose of drug compared to control (41 mg/ml). The result more revealed that there was significant increase in IgM level with all treatments compared to saline treated mice (12 mg/ml). Increase in IgM levels was depend on the dose of drug, Diclo treatment increased IgM concentration from 28 to 31 (mg/ml) and administration of Para augmented IgM level from 12.3 to 34 (mg/ml), however, Ibu treatment diminished the concentration from 52 to 32.2 (mg/ml) (Fig. 3-B).

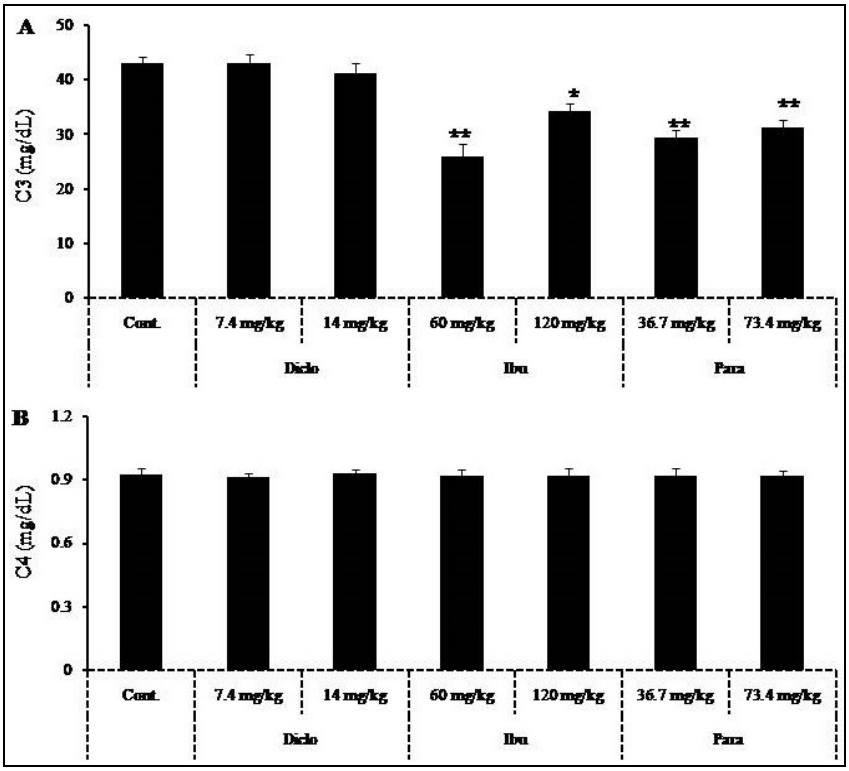

Figure 4. Changes in C3 and C4 concentration in PB after repeated administration of different pain killers. Mice treated with saline (control), Diclo 1 (7.4 mg/kg), Diclo 2 (14.8 mg/kg), Para 1 (36.7 mg/kg), Para 2 (73.4 mg/kg), Ibu 1 (60 mg/kg), Ibu 2 (120 mg/kg) intra-peritoneally (i.p.) daily for one month. Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

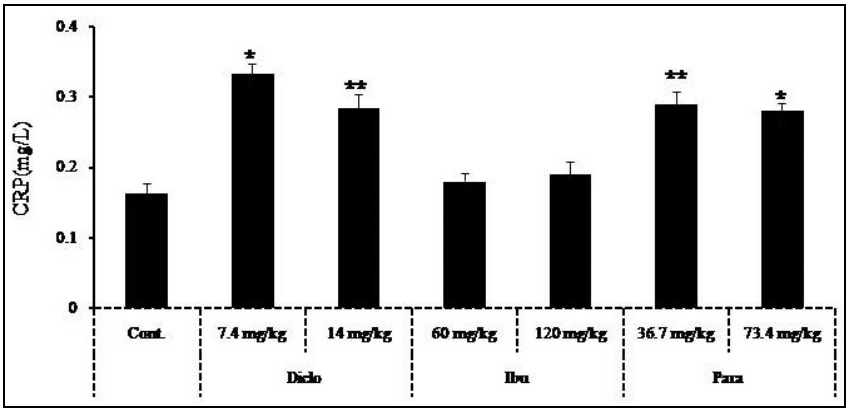

Figure 5. Changes in CRP concentration in PB after repeated administration of different pain killers. Mice treated with saline (control), Diclo 1 (7.4 mg/kg), Diclo 2 (14.8 mg/kg), Para 1 (36.7 mg/kg), Para 2 (73.4 mg/kg), Ibu 1 (60 mg/kg), Ibu 2 (120 mg/kg) intraperitoneally (i.p.) daily for one month. Data were represented as mean ± SE (n = 3). *: Statistically significant comparison of control group and other treated groups ($p < 0.05$), **: Highly significant ($p < 0.01$).

In figure 4-A, Ibu (60 mg/kg and 120 mg/kg) and Para (36.7 mg/kg and 73.4 mg/kg) treatments had significantly reduced the concentration of complement component C3; while administration of Diclo revealed a non-significant decrease in complement C3 levels depending on the dose of Diclo when compared to control mice. Further, all examined treatments did not differ significantly from the control mice in terms of complement C4 (Fig. 4-B). Moreover, Ibu (60 mg/kg and 120 mg/kg) treatments slightly elevated CRP concentration in PB, while Diclo (7.4 mg/kg and 14.8 mg/kg) and Para-treated (36.7 mg/kg and 73.4 mg/kg) mice indicated significant increase in comparison to saline-treated group (Fig. 5).

4. DISCUSSION

NSAIDs are considered as a group of the most abused drugs by mains of combining the pharmacological actions of anti-inflammatory and analgesia, so they can easily be bought over the counter [38]. Alternative to NSAIDs, Para is recommended as a first-line treatment option for mild to moderate chronic pain [39] giving analgesia by raising the pain threshold, chiefly through a central rather than peripheral mechanism [40]. Because NSAIDs and paracetamol are commonly used, we thought it is important to investigate their adverse effects on immunological and hematological parameters.

Alternations in the organ-body weight ratio may be a marker of cell constriction or inflammation and this constriction may occur as a result of lack of fluid from the organ related to damage, however an increase in organ-body weight ratio may refer to inflammation [41]. Further, numerous drugs are supposed to cause immunotoxic effects in humans and animals leading to disorders in the immune system that observed by alternations in immune related organs (spleen and thymus) weight [42]. Current results revealed all tested drugs caused non-significant change in body weight, the relative and absolute weight of liver and kidney, however there were adverse effects on the relative and absolute weight of lymphoid organs (spleen, thymus and lymph nodes). Similar results were speculated by Oyedeji et al. [43] who reported that Para caused non-significant changes in the body weight of rats post treatment for 42 days and analysis of organ weight in toxicological studies is an important endpoint for recognition of potentially deleterious effects of chemicals [44] that may occur in the absence of any morphological changes [45].

The present study showed that Diclo administration (14.8 mg/kg) caused decrease in RBCs count, Hb content and Hct%, despite there was no effect with Diclo at dose of 7.4 mg/kg concluding that it is dependent on dose toxicity. While Para and Ibu treatment showed increase in RBCs count, Hb content and Hct%. There was no change in platelets count with Para administration; however, Ibu and Diclo treatment presented elevation in their count. These results are in line with those of Thanagari et al. [46], El-Maddawy and El-Ashmawy [47] and Orinya et al. [48], who reported that Diclo induced highly significant decrease in Hb, PCV values resulting anemia that may refer to loss of blood during gastrointestinal bleeding that induced by diclofenac sodium. Moreover, chronic use of Ibu could affect hematological functions and time of exposure may promote ibuprofen toxicity depending on dose [49]. In addition, Para overdose causes liver damage based on the dose and this damage caused alterations in the red blood cell count, and packed cell volume [50, 51]. Para has the potential to inhibit erythropoietin release from the kidneys [52] resulting in the reduction in erythrocytes production, Hb concentration and Ht value and this may lead to anaemia. Further, the decrease in hematological parameters caused by Para may be attributed to the hyper-activity of bone marrow leading to the production of red blood cells with impaired integrity that are easily destroyed in the circulation [53].

NSAIDs have immunomodulatory effects by interfering with human T lymphocyte activation, proliferation and cytokine synthesis [54-56] through inhibition of Cox activity. Cox-2 is expressed in activated B lymphocytes that are required for optimal antibody production predicting that NSAID therapy can have reverberations on antibody synthesis [57, 58]. The current data revealed that by the end of treatment, there were significant down-regulated activities of IgG in response to the examined drugs; however, IgM synthesis was enhanced with all tested drugs. In agreement with the present results, Bancos et al. [59] revealed that a panel of commonly used NSAIDs dulls antibody

synthesis in human peripheral blood mononuclear cells (PBMCs) and in purified B cells. Moreover, ibuprofen's ability to diminish antibody production was dependent on concentration- and time and probably occurred via Cox-2 inhibition, as Cox-2 is responsible for ibuprofen-mediated IgG, but not IgM inhibition. In addition, Diclo forms neoantigens with RBCs that may induce the production of autoantibodies and drug-dependent antibodies [60] leading to the production of antibodies against RBCs and/or platelets [61].

Complement proteins are direct contributors in the maintenance of cellular turnover, healing, proliferation, regeneration and tissue integrity [62]. The results obtained herein revealed that Para or Ibu administration reduced levels of complement component C3 not C4 in serum, whereas Diclo treatment had a non-significant decrease in complement C3 levels in dose dependent manner and no effect on C4 level suggesting that the tested drugs activated the alternative complement pathway that relies on C3 not C4 leading to reduction of C3 level in serum. Our findings have been supported by Prohászka et al. [63] and Navratil et al. [64] who reported that hepatocytes changes or damage induced by paracetamol treatment is required for complement activation. In addition, complement components contribute in host tissue injury in several clinical conditions, and they are activated during hepatocytes regeneration for hepatoprotection through activation of C3 that is required for a normal hepatic regenerative response [65]. Further, the alternative complement pathway is activated, and may associate with deleterious reactions contributed to NSAID such as acute tubular injury induced by NSAID leading to acute kidney injury [66]. Moreover, some drugs like NSAIDs may directly stimulate effector mechanisms, such as the complement system by direct modulation of arachidonic acid pathway [67].

In the present study, a marked increase in CRP level was recorded in the sera of mice treated with Diclo, Ibu or Para for one month suggesting that continuous NSAIDs use may revert their effects on CRP levels in serum. These results were similar to those of Tarp et al. [68] who revealed the cyclooxygenase 2-selective NSAID lumiracoxib was associated with a significant increase in the CRP level and NSAIDs use for longer periods of time can lead to severe health problems like mucosal ulceration and inflammation in the lower gastrointestinal (GI) tract [69] that may be associated with elevation in CRP level [70]. Further, Para is recognized to have trifling anti-inflammatory effect and its overdose is linked with inflammation that marked by an increase in the inflammatory cytokines [71, 72].

5. CONCLUSION

From the present study, it is concluded that daily administration of Diclo, Ibu, or Para for one month caused adverse effects on hematological parameters (RBCs, HB contents, HT% and Plts counts), and they caused immunomodulatory effects on levels of IgG and IgM, in addition to perturbations in immune related organs (spleen, bone marrow and lymph node). These drugs also induced an increase in CRP level in serum and enhanced activation of alternative complement system that may contribute to deleterious reactions induced by tested drugs suggesting that continuous use of Diclo, Ibu, or Para may lead to development of haematotoxicity and immunotoxicity. So caution needs to be exercised in these drugs administration, which should be limited to the lowest therapeutic doses, to prevent its harmful effect. Further studies are needed to assess the relationships between administration of Diclo, Ibu or para and immunological and hematological perturbations.

Abbreviations

Para: Paracetamol; Diclo: Diclofenac; Ibu: Ibuprofen; COX: Cyclooxygenase; NSAIDS: non-steroidal anti-inflammatory drugs; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

ETHICS APPROVAL

All animal experimentation protocols were carried out in agreement with the Ethical Principles for Animal Research established by Egyptian National Research Center, Cairo, Egypt.

FUNDING

Not applicable.

TRANSPARENCY DECLARATION

The author declares that she has no competing interests.

REFERENCES

1. Nalamachu S. An overview of pain management: the clinical efficacy and value of treatment. Am J Managed Care. 2013; 19(14): 261-266.
2. Milsom I, Minic M, Dawood MY, Akin MD, Spann J, Niland NF, Squire RA. Comparison of the efficacy and safety of nonprescription doses of naproxen and naproxen sodium with ibuprofen, acetaminophen, and placebo in the treatment of primary dysmenorrhea: a pooled analysis of five studies. Clin Ther. 2002; 24:1384-1400.
3. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev. 2004; 56: 387-437.
4. Shafi M, Garg UK, Saqib N, Baba OK, Farid BD, Wali A. Haemato-biochemical studies on diclofenac, ibuprofen and nimesulide induced toxicity in broilers. Nat Env Poll Tech. 2012; 11(4): 649-652.
5. Zarghi A, Arfaei S. Selective COX-2 inhibitors: a review of their structure-activity relationships. Iran J Pharmac Res. 2011; 10(4): 655-683.
6. Bushra R, Aslam N. An overview of clinical pharmacology of ibuprofen. Oman Med J. 2010; 25(3): 155-161.
7. Reynolds EF. Aspirin and similar analgesic and anti-inflammatory agents. In: Martindale. The Extra Pharmacopoeia, 28th edn., Pharmaceutical Press: London, 1982: 234-282.
8. Hörl WH. Non-steroidal Antiinflammatory Drugs and the kidney. Pharmaceuticals. 2010; 3: 2291-2321.
9. Bradbury F. How important is the role of the physician in the correct use of a drug? An observational cohort study in general practice. Int J Clin Pract Suppl. 2004; 144: 27-32.
10. Capone ML, Tacconelli S, Di-Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat. 2007; 82(1-4): 85-94.
11. Traversa G, Walker AM, Ippolito FM, Caffari B, Capurso L, Dezi A, et al. Gastroduodenal toxicity of different nonsteroidal antiinflammatory drugs. Epidemiol. 1995; 6(1): 49-54.
12. Higuchi K, Umegaki E, Watanabe T, Yoda Y, Morita E, Murano M, et al. Present status and strategy of NSAIDs-induced small bowel injury. J Gastroenterol. 2009; 44(9): 879-888.
13. Bennett W, Henrich WL, Stoff JS. The renal effects of nonsteroidal anti-inflammatory drugs: Summary and recommendations. Am J Kidney Dis. 1996; 28: 56-62.
14. Aprioku JS and Uche FI. Renal Effects of non-steroidal antiinflammatory drugs in albino rats. Br J Pharm Res. 2013; 3(3): 314-325.
15. Fries JF. Assessing and understanding patient risk. Scand J Rheumatol. 1992; 92: 21-24.
16. Orinya OA, Adenkola AY, Ogbe RJ. Haematological and biochemical studies on the effect of diclofenac sodium on Wistar *Rattus norvegicus*. Int J Pharm Chem Biol Sci. 2016; 10(5): 2231-2242.
17. Boshra SA, Hussein MA. The protective role of colchicine on diclofenac sodium induced hepatorenal toxicity in albino rats model. Int J Pharm Sci Res. 2014; 5(12): 5136-5144.
18. Ahmad I, Qureshi TA, Khan FA, Mughal SAK, Sadique U, Shah Z, et al. Evaluation of biochemical effects of diclofenac sodium in goats. J Anim Plant Sci. 2012; 22(2): 1-4.
19. Oaks JL and Khan A. Diagnostic investigation of vulture mortality: the anti-inflammatory drug diclofenac is associated with visceral gout. J Ind Vet. 2004; 23: 152-158.
20. Bhogaraju A, Nazeer S, Al-Baghdadi Y, Rahman M, Wrestler F, Patel N. Diclofenac-associated hepatitis. J South Mediterian. 1999; 7: 711-713.
21. Aydin G, Gokcimen A, Oncu M, Clcek E, Karahan N, Golkalp O. Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. Turk J Vet Anim Sci. 2003; 27: 1131-1140.
22. El-Maddawy ZKH, El-Ashmawy IM. Hepato-renal and haematological effects of diclofenac sodium in rats. Global J Pharmacol. 2013; 7(2): 123-132.
23. Anderson MD, Piper SE and Swan GE. Non-steroidal anti-inflammatory drug use in South Africa and possible effects on vultures. S Afr J Anim Sci. 2005; 101: 112-114.
24. Graham GG, Scott KF. Mechanism of action of paracetamol. Am J Ther. 2005; 12(1): 46-55.
25. Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent

pharmacological findings. Inflammopharmacology. 2013; 21(3): 201-232.

26. Smith HS. Potential analgesic mechanisms of acetaminophen. Pain Physician. 2009; 12: 269-280
27. Jóźwiak-Bebenista M, Nowak JZ. Paracetamol: mechanism of action, applications and safety concern. Acta Pol Pharm. 2014; 71(1): 11-23.
28. Chiam E, Weinberg L, Bellomo R. Paracetamol: a review with specific focus on the haemodynamic effects of intravenous administration. Heart Lung Vessel. 2015; 7(2): 121-132.
29. McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Acetaminophen-induced liver injury in rats and mice: comparison of protein adducts mitochondrial dysfunction, and oxidative stress in the mechanism of toxicity. Toxicol Appl Pharmacol. 2012; 264(3): 387-394.
30. Seham A, Abd E, Begonia M, Morales A, Sara K, Daniel LS, Lucy FD. Localisation of cyclooxygenase-3 in rat central nervous system. Univ Cambridge J Physiol. 2004; 555P: C156.
31. European community regulations. Diclofenac sodium, Safety Data Sheet, 2008.
32. Adams SS, Bough RG, Cliffe EE, Lessel B, Mills RF. Absorption, distribution and toxicity of ibuprofen. Toxicol Appl Pharmacol. 1969; 15(2): 310-330.
33. European community regulations. Acetaminophen Safety Data Sheet, 2008.
34. Arce S, Nawar, HF, Muehlinghaus G, Russell MW, Connell TD. In vitro induction of immunoglobulin A (IgA)-and IgM-secreting plasma blasts by cholera toxin depends on T-cell help and is mediated by CD154 up-regulation and inhibition of gamma interferon synthesis. Infect Immun. 2007; 75(3): 1413-1423.
35. Keggan A, Freer H, Rollins A, Wagner B. Production of seven monoclonal equine immunoglobulins isotyped by multiplex analysis. Vet Immunol Immunopathol. 2013; 153(3): 187-193.
36. Aniagu SO, Nwinyi FC, Akumka DD, Ajoku GA, Dzarma S, Izebe KS, et al. Toxicity studies in rats fed nature cure bitters. Afr J Biotechnol. 2005; 4(1): 72-78.
37. Tukmechi A, Rezaee J, Nejati V, Sheikhzadeh N. Effect of acute and chronic toxicity of paraquat on immune system and growth performance in rainbow trout, *Oncorhynchus mykiss*. Aqua Res. 2014; 45(11): 1737-1743.
38. Gilman A, Goodman L, Gilman A. The Pharmacological basis of therapeutics. 6th edn Macmillian Pub. Co. Inc. New York. 1990.
39. Ripamonti CI, Bandieri E, Roila F. Management of cancer pain: ESMO Clinical Practice Guidelines. Ann Oncol. 2011; 22(6): 69-77.
40. Raffa RB, Stone DJ, Tallarida RJ. Unexpected and pronounced antinociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine. Eur J Pharmacol. 2001; 412(2): R1-2.
41. Moore K, Dalley A, Agur, AMR. Clinically orientated anatomy. 4th edn. Lippincott Williams and Williams, Philadelphia. 1999: 263-271.
42. Descotes J. Pseudo-allergic drug reactions. Clin Res Pract Drug Reg Affairs. 1986; 4(1): 75-84.
43. Oyedeji KO, Bolarinwa AF, Jeniran SS. Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats. Res J Pharmacol. 2013; 7(2): 21-25.
44. Nirogi R, Goyal VK, Jana S, Pandey SK, Gothi A. What suits best for organ weight analysis: review of relationship between organ weight and body/brain weight for rodent toxicity studies. Int J Pharm Sci Res. 2014; 5(4): 1525-1532.
45. Bailey SA, Zidell RH, Perry RW. Relationships between organ weight and body/brain weight in the rat: What is the best analytical endpoint? Toxicol Pathol. 2004; 32(4): 448-466.
46. Thanagari BS, Fefar DT, Prajapati KS, Jivani BM, Thakor KB, Patel JH. Haemato-biochemical alterations induced by diclofenac sodium toxicity in Swiss albino mice. Vet World. 2012; 5: 417-419.
47. El-Maddawy ZKH, El-Ashmawy IM. Hepato-renal and haematological effects of diclofenac sodium in rats. Global J Pharmacol. 2013; 7(2): 123-132.
48. Orinya OA, Adenkola, AY, Ogbe RJ. Haematological and biochemical studies on the effect of diclofenac sodium on Wistar *Rattus norvegicus*. Int J Biol Chem Sci. 2016; 10(5): 2231-2242.
49. Aprioku JS, Nwidu LL, Amadi CN. Evaluation of toxicological profile of ibuprofen in Wistar albino rats. Am J Biomed Sci. 2014; 6(1): 32-40.
50. Al-Saady MAJ, Abdul-Latif A, Al-Shemmery HN. Pharmacological effects of diclofenac sodium on some haematological parameters of male rabbits. Med J Babyl. 2011; 8(3): 441-452.
51. Samuel SA, Francis AO, Ayomide O, Onyinyechi UO. Effects of paracetamol-induced liver damage on some hematological parameters: red blood cell (RBC) count, white blood cell (WBC) count, and

packed cell volume (PCV) in wistar rats of either sex. Indo Am J Pharm Res. 2015; 5(7): 2593-2599.

52. Dwivedi V, Mishra J, Shrivastava A. Efficacy study of livartho against paracetamol induced hepatotoxicity in adult Sprague Dawley rats. J Drug Metab Toxicol. 2015; 5: 175-181.
53. Adeneye AA, Ajagbonna OP, Adeleke TI, Bellow SP. Hematological evaluation of methanol seed extract of citrus. J Ethnopharmacol. 2006; 105: 374-379.
54. Inigues M, Punzon C, Fresno M. Induction of cyclooxygenase-2 on activated T lymphocytes: regulation of T cell activation by cyclooxygenase-2 inhibitors. J Immunol. 1999; 163: 111-119.
55. Paccani SR, Boncristiano M, Ulivieri C, D'Elios MM, Del Prete G, Baldari CT. Nonsteroidal anti-inflammatory drugs suppress T-cell activation by inhibiting p38 MAPK induction. J Biol Chem. 2002; 277: 1509-1513.
56. Hartel C, von Puttkamer J, Gallner F, Strunk T, Schultz C. Dose-dependent immunomodulatory effects of acetylsalicylic acid and indomethacin in human whole blood: potential role of cyclooxygenase-2 inhibition. Scand J Immunol. 2004; 60: 412-420.
57. Ryan EP, Pollock SJ, Murant TI, Bernstein SH, Felgar RE, Phipps RP. Activated human B lymphocytes express cyclooxygenase-2 and cyclooxygenase inhibitors attenuate antibody production. J Immunol. 2005; 174: 2619-2626.
58. Bernard MP, Phipps RP. CpG oligodeoxynucleotides induce cyclooxygenase-2 in human B lymphocytes: implications for adjuvant activity and antibody production. Clin Immunol. 2007; 125: 138-148.
59. Bancos S, Bernard MP, Topham, DJ, Phipps RP. Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. Cell Immunol. 2009; 258(1): 18-28.
60. Salama A, Kroll H, Wittmann G, Mueller-Eckhardt C. Diclofenac-induced immune haemolytic anaemia: simultaneous occurrence of red blood cell autoantibodies and drug-dependent antibodies. Brit J Haematol. 1996; 95(4): 640-644.
61. Meyer O, Hoffmann T, Aslan T, Ahrens N, Kiesewetter H, Salama A. Diclofenac-induced antibodies against RBCs and platelets: two case reports and a concise review. Transfusion. 2003; 43(3): 345-349.
62. Rutkowski MJ, Sughrue ME, Kane AJ, Ahn BJ, Fang S, Parsa AT. The complement cascade as a mediator of tissue growth and regeneration. Inflamm Res. 2010; 59(11): 897-905.
63. Prohászka Z, Singh M, Nagy K, Kiss E, Lakos G, Duba J, Füst G. Heat shock protein 70 is a potent activator of the human complement system. Cell Stress Chaperones. 2002; 7(1): 17-22.
64. Navratil JS, Liu CC, Ahearn JM. Apoptosis and autoimmunity. Immunol Res. 2006; 36: 3-12.
65. Markiewski MM, DeAngelis RA, Strey CW, Foukas PG, Gerard C, Gerard N, Lambris JD. The regulation of liver cell survival by complement. J Immunol. 2009; 182(9): 5412-5418.
66. Clark A, Weymann A, Hartman E, Turmelle Y, Carroll M, Thurman JM, et al. Evidence for non-traditional activation of complement factor C3 during murine liver regeneration. Mol Immunol. 2008; 45(11): 3125-3132.
67. Palviainen MJ, Junnikkala S, Raekallio M, Meri S, Vainio O. Activation of complement system in kidney after ketoprofen-induced kidney injury in sheep. Acta Vet Scand. 2015; 57: 15-20.
68. Tarp S, Bartels EM, Bliddal H, Furst DE, Boers M, Danneskiold-Samsøe B, Christensen R. Effect of nonsteroidal antiinflammatory drugs on the C-reactive protein level in rheumatoid arthritis: A meta-analysis of randomized controlled trials. Arthritis Rheum. 2012; 64(11): 3511-3521.
69. Sostres C, Gargallo CJ and Lanas A: Nonsteroidal anti-inflammatory drugs and upper and lower gastrointestinal mucosal damage. Arthritis Res Ther. 2013; 15(3): 3.
70. Tomizawa M, Shinozaki F, Hasegawa R, Shirai Y, Motoyoshi Y, et al. Elevated C-reactive protein level predicts lower gastrointestinal tract bleeding. Biomed Rep. 2016; 4(6): 711-714.
71. Ghosh J, Das J, Manna P, Sil PC. Acetaminophen induced renal injury via oxidative stress and TNF-alpha production: therapeutic potential of arjunolic acid. Toxicology. 2010; 268: 8-18.
72. Jaeschke H, Williams CD, McGill MR, Xie Y, Ramachandran A. Models of drug-induced liver injury for evaluation of phytotherapeutics and other natural products. Food Chem Toxicol. 2013; 55: 279-289.