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Immunological, Hematological and Biochemical Effects of Aspirin in Low and High Doses in Male Albino Rats

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Abstract

This study aimed to investigate the immunological, hematological and biochemical effects of low and high doses of aspirin in male albino rats and to clarify the modulatory influence of vitamin C on the negative impacts of aspirin. Thirty five (35) adult male albino rats (140-160 g) have been split into five groups. The first group (G1) was deemed a control group, wherein 1 ml/kg of saline solution was given to rats. A low dose of aspirin (0.2 mg/kg) alone was administered orally to the second group (G2). A high dose of aspirin (0.6 mg/kg) was administered orally to the third group (G3). The forth group (G4) received oral treatment with a low dose of aspirin and vitamin C (200 mg/Kg). A high dose of aspirin, in combination with vitamin C (200 mg/kg), was given to the fifth group (G5). The findings obtained at the end of the experimental duration (four weeks) showed that the low and high doses of aspirin caused significant rises in serum IgG, monocytes count, RBCs count, blood Hb, PCV%, serum creatinine, AST and ALT and significant decreases in serum IgM, neutrophils count, lymphocytes count and AFT. The low dose of aspirin did not induce significant changes in MDA, SOD, HDL-C, TC and TP levels, While, aspirin in the high dose only induced significant increases in TC and MDA levels, and, significant decreases in SOD, HDL-C and TP levels. Moreover, aspirin at both doses did not alter the total WBCs count, serum IgE, serum IgA, platelets count and LDL-C levels relative to the control group. Vitamin C administration along with aspirin reduced the immunological, hematological and biochemical alterations induced by aspirin. Conclusion: Aspirin in either low or high doses induced disturbances in both humoral and cell-mediated immunity and alterations in hematological parameters and caused adverse effects in liver and kidney functions. Moreover, the two doses of aspirin did not exhibit carcinogenic or anemic effects and the side effects of aspirin were dose-dependent as the low dose had less adverse effects than the high dose. Vitamin C supplements exhibited immunomodulatory effects and could counteract the toxic effects of aspirin intake.

Keywords: Aspirin, vitamin C, immunoglobulins, hematological parameters, liver and kidney functions, albino rats.

INTRODUCTION

An immune system that defends the body against infection is a complicated network of cells and proteins. Leukocytes (WBCs) are the second arm of the innate immune system, functioning like autonomous, single-celled organisms. The number of leukocytes in the blood is often an indicator of disease. Leukocytosis is the rise in the number of leukocytes above the upper limit. When it is part of a healthy immune response that occurs frequently, it is normal. Leukopenia is considered as a reduction below the lower limit. It reveals a weakened immune system (Mohammad *et al*., 2020). The main players in innate immunity are

neutrophils. A range of inflammatory cytokines and chemokines are generated by the intervention of mitogen-activated protein kinase (MAPK) pathways following neutrophil extravasation (adhesion, transmigration, chemotaxis, etc.) from the circulatory system to the infection site. In addition to sharing a defensive alliance with neutrophils in innate immunity, monocytes/macrophages are also involved in the regulation of an adaptive-immune assault (Cloutier *et al*., 2007).

Immunoglobulins (Igs) are big glycoproteins formed by B-cells that are main elements of the humoral immune system that protect against invasive antigens. In clinical practice, serum immunoglobulin rates are regularly calculated as they provide essential data on humoral immune status. Some humoral immunodeficiencies are characterized by low levels of immunoglobulin (Alvarez *et al*., 1999). In comparison, elevated levels of immunoglobulin (polyclonal gammopathy) are seen in liver diseases, hematological disorders, chronic inflammatory diseases, infections and malignancies. In addition, the level of immunoglobulin helps to diagnose certain diseases, especially liver diseases (Martin *et al*., 1984).

Aspirin (acetylsalicylic acid), one of the most frequently prescribed non-steroidal anti-inflammatory drugs (NSAIDs), is probably the most highly used prescription medication in the world. It has been utilized because of its anti-inflammatory, antipyretic and analgesic impact in different pathological cases. In addition to its well-known positive anti-inflammatory impact in the cardiovascular system and its work in anti-cancer treatment, it also has a large influence on the role of immune cells, including NO production, cytokines (e.g. IL-1β, IL-6, IL-8, TNF-a) and the expression of the adhesion molecule. Several new and fascinating informations on the function of aspirin in immune system cells and some immunopathological states have emerged. Aspirin, for example, causes tolerogenic activity in dendritic cells and defines the fate of naive T cells in regulatory phenotypes, suggesting its immune-regulatory capacity for immune tolerance (Desborough *et al*., 2017).

On the other hand, different lines of evidence suggested that aspirin can suppress the antibody-mediated humoral immune response and may reduce the host defense which include: decreased synthesis of IgM and IgG antibodies in activated human peripheral blood mononuclear cells (PBMCs) (Bancos *et al*., 2009); Progressive decrease in serum IgA levels in patients with juvenile rheumatoid arthritis (Kondo *et al*., 1993). Aspirin and its derivatives can also produce immunosuppressive impact by decreasing the response of neutrophilic extravasation associated with innate immunity. Moreover, previous studies indicated a substantial reduction in the percentage and number of peripheral CD4+ T cells in BALB/c mice treated with low or high doses of aspirin for four successive weeks, moreover, aspirin has been described to induce apoptosis in lymphocytes (Javeed *et al*., 2009).

A significant number of clinical studies link aspirin to blood disorders such as anemia and cytopenia. Although the relative risk of aspirin blood disorders is considered low, there have been substantial death rates owing to aspirin-induced blood disorders (Navratil *et al.*, 1992). While aspirin-using blood disorders have been clinically reported, comparatively little experimental trials have been undertaken to explain and confirm the correlation. Literature results, on the other hand, indicate mortality and morbidity with adverse impacts of aspirin on multiple organ systems. In addition, the occurrence of gastrointestinal ulceration, hepatotoxicity, nephrotoxicity and even renal cell cancer is associated with long-term therapeutic use of aspirin (Pignatelli *et al*., 2008 and Jahan *et al.*, 2019).

One of the best nutrients is vitamin C or ascorbic acid, which is the most plentiful and efficient antioxidant in the human body. Vitamin C can help protect against immune system defects, cardiovascular disease, prenatal health complications, eye illness and even the skin. Vitamin C's antioxidant role is linked to its reversible properties of oxidation and reduction. Vitamin C can, therefore, partly prevent some forms of damage to hepatic and renal cells (Dowell and Second, 1989, Sies *et al*., 1992 and Netke *et al*., 1997 and Ergul *et al.,* 2010).

The purpose of this research was to examine the impact of aspirin in low and high doses on immunological, hematological and biochemical parameters in healthy male albino rats, and to determine the modulatory impact of vitamin C on alterations caused by aspirin administration.

MATERIAL AND METHODS

Experimental animals:

Thirty five (35) male albino rats from Sprague Dawley strain (Rattus norvegicus) have been used in the present study. Their weights ranged from (160 - 180 grams). The animals were obtained from the research center for the animal in Cairo. They were supplied with appropriate food for rat that is composed of (150 g of corn starch, 10 % of corn oil, 10 % of casein, 5 % of cellulose and 4% of salt) and tap water. Animals were allowed to acclimate for two weeks in the Animal House; Faculty of science, Minia University with a photoperiod (dark light cycle 12h: 12h) and the temperature was adjusted at 20-25°C before beginning the experiment.

Drugs:

Aspirin and vitamin C (L-ascorbic acid) tablets were purchased from "Outpatient Pharmacy. The two drugs were powdered separately in a glass mortar. Aspirin was dissolved in saline solution (0.9 % Nacl) and two doses of aspirin were prepared and used in the present study, the low dose was (50 mg/kg b. wt.) (Scholer *et al*., 1986) and the high dose was (150 mg/kg b. wt.) (Mc Quay and Moore, 2007). Vitamin C was dissolved in aqueous solution using distilled water to obtain 10% of the stock solution. The prepared vitamin C concentration for the test was 200 mg/kg (Aksoy *et al.,* 2oo5). All additional chemical reagents and kits included in the study were of standard analytical grades and were obtained from Sigma Chemical Co. (St. Louis, O, USA).

Experimental design:

The rats were split into five groups, after the adaptation period, as follows:

Group 1: Regarded to be a control group in which 1 ml/kg of saline solution (0.9% Nacl) was administered orally and daily to rats for 28 days.

Group 2: It acted as the low aspirin group where the low aspirin dose (50mg/kg) was administered orally and daily to rats for 28 days.

Group 3: The high dose aspirin group wherein the high dose of aspirin (150 mg/kg) was administered daily and orally to rats for 28 days.

Group 4: this group received oral and daily treatment with the low dose of aspirin (50 mg/kg) and vitamin C (200 mg/kg) for 28 days.

Group 5: that administered the high dose of aspirin (150 mg/kg) and vitamin C (200 mg/kg) daily and orally for 28 days.

Immunological, Hematological and biochemical analysis:

Animals were fasted overnight at the end of the experimental duration, however, free access to water was permitted. They were slaughtered under diethyl ether anesthesia and two blood samples were taken. The first sample was taken for immunological and hematological tests in the heparinized tube (2,25µ heparin/5 ml of blood). The second sample was gathered for serum separation in non-heparinized tubes. To estimate certain immunological and biochemical variables, the tubes were centrifuged at 3000 rpm for 15 min. Serum IgG and IgE levels were determined by an enzyme-linked immunosorbent assay (ELISA) according to the method of (Chang *et al*, 2009). A commercial assay using the BN-II device (Dade Behring, Marburg, Germany) was performed to measure serum IgM and IgE levels according to the method of (Schmid *et al*, 2005). Differential and the total number of leukocytes and all hematological parameters have been calculated using the Micros ABX automated cell counter (Roche Diagnostic System, Montpellier, France) (Beausoleil *et al*., 2010). The following hematological variables have been analyzed: hemoglobin, packed cell volume, erythrocytes count, platelets number and the differential and the total number of leukocytes. The levels of serum alanine and aspartate aminotransferase (ALT&AST) were calculated by the method of (Reitman and Frankel, 1957). Malondialdehyde (MDA) in liver tissue was calculated using the Uchiyama and Mihara method (1978) and the level of Superoxide dismutase (SOD) was measured using the Marklund and Marklund method (1974). Enzymatic colorimetric methods were used to determine high-density lipoprotein cholesterol (HDLC) (Kostener, 1977). The spectroscopic determination of total cholesterol in serum was performed using the method of (Allain *et al.,* 1974). Low-density lipoprotein cholesterol (LDL-C) has been measured using the method of (Fruchart, 1982). The total protein of the serum was calculated using the method defined by the (Bishop *et al.,* 2000). Serum creatinine was calculated using the method defined by (Schirmeister, 1964).

Statistical analysis:

Statistical analysis of the current data was conducted during the one-way variance analysis (ANOVA test). The mean± standard error (M± SE) was used to express the findings. The significance of the student "t" test was used and variations were deemed to be significant at ($P \leq 0.05$), extremely significant at ($P \leq 0.01$) and very extremely significant at ($P \leq 0.001$).

RESULTS

Serum immunoglobulin levels like total IgG, total IgM, total IgE, and total IgA in the control and treatment groups were measured and are shown in the Table (1). It was clear that there was a significant rise ($p<0.05$) in IgG level after administration of low or high doses of aspirin as compared to control. Co-administration of the low and high doses of aspirin with vitamin C significantly decreased serum IgG level ($p<0.05$). Both low and high doses of aspirin substantially reduced the serum IgM level (($p<0.01$ and $p<0.001$) versus the control level. The combined administration of low or high doses of aspirin and vitamin C induced a substantial rise ($p<0.05$) in total IgM levels relative to low or high doses of aspirin alone treated groups. On the other hand, neither low nor high dose of aspirin alone or in combination with vitamin C induced significant changes ($p>0.05$) in serum total IgE or total IgA levels.

A total white blood cell (WBCs), lymphocytes, monocytes, and neutrophils counts in control and treated groups were calculated and the results are shown in the table (2). Administration of aspirin in high and low doses did not induce significant effect on (WBCs) count ($p>0.05$). Co-administration of vitamin C with the high and low doses of aspirin

significantly ($p<0.05$) increased (WBCs) count. Administration of low or high doses of aspirin significantly ($p<0.05$) reduced lymphocytes count relative to the control group. The combined administration of low or high doses of aspirin along with vitamin C induced a significant increase ($p<0.05$) in lymphocytes count. However, the results showed a significant increase ($p<0.05$) in monocytes count after administration of low or high doses of aspirin as compared to control. This effect was not altered significantly ($p>0.05$) in co-administration of vitamin C with low or high aspirin doses. Aspirin in low or high doses showed significant decline ($p<0.05$) in neutrophils count relative to the control group. Co-administration of vitamin C with aspirin exhibited significant increase ($p<0.05$) in neutrophils count for rats taken low dose of aspirin only.

Table 1: Impact of low or high doses of aspirin alone and in combination with vitamin C on the levels of four types of immunoglobulin (IgG, IgM, IgE and IgA) in serum of male albino rats (means± SE).

Parameter / group	IgG (mg/dl)	IgM (mg/dl)	IgE (IU/ml)	IgA (mg/dl)
G1: Control	55.4±0.0	33±0.0	3.2±0.0	16.2±0.0
G2: Aspirin (low)	83.7±5.2*	14.2±1.95**	2.7±0.09	15.9±2
G6: Asp.(low) +vitamin	60±2#	29.1±4.7#	3.2±0.5	17.6±1.1
G3: Aspirin (high)	77.5±4.8*	12.5±0.9***	2.7±0.06	18.5±0.5
G7: Asp.(high) +vitamin	62.8±1.5#	18.9±0.9#	2.6±0.1	22±2

Increase or decrease significantly relative to the control group (* P<0.05, ** P<0.01, *** P<0.001).
Increase or decrease significantly relative to aspirin group (# P<0.05, ## P<0.01, ### P<0.001).

Table 2: Impact of low or high doses of aspirin alone and in combination with vitamin C on total leucocytes, lymphocytes, monocytes and neutrophils levels in male albino rats (means±SE).

Parameter / Group	Leucocytes ($x10^9$/L)	Lymphocytes ($x10^9$/L)	Monocytes ($x10^9$/L)	Neutrophils ($x10^9$/L)
G1:Control	8.5± 0.0	66.3±1.9	2.4±0.1	33.4±2.1
G2: Aspirin (low)	6.9±3.7	47.9±2.1*	16±4.3*	15.7±3.6*
G6: Asp.(low) + vitamin	10.5±0.6#	60.3 ±13#	17.7±2.4	22±13#
G3: Aspirin (high)	7.15±0.7	53.7±1.8*	15.9±1.6*	14.5±3.8*
G7: Asp.(high)+ vitamin	10.6±0.7#	71.4±2.4#	16.7±0.96	18±0.37

Increase or decrease significantly relative to the control group (* P<0.05, ** P<0.01, *** P<0.001).
Increase or decrease significantly relative to aspirin group (# P<0.05, ## P<0.01, ### P<0.001).

Total RBCs count, hemoglobin (Hb) content, packed cell volume (PCV) and Platelets number were calculated in the control and treatment groups and the findings are shown in the table (3). Aspirin in high or low doses have shown a significant rise in total RBCs count ($p<0.01$ and $p<0.001$) relative to the control group respectively. Co-administration of vitamin C at low or high doses of aspirin substantially decreased ($p<0.01$ and $p<0.001$) RBCs count. Moreover, there was significant increase ($p<0.01$) in Packed cell volume (PCV) and hemoglobin content of rats administered low or high doses of aspirin relative to the control group. Co-administration of vitamin C with low or high doses of aspirin significantly

($p<0.05$) decreased these parameters. On the other hand, no significant changes occurred in platelets number of rats taken low or high doses of aspirin either alone or in conjunction with vitamin C.

Table 3: Impact of low or high doses of aspirin alone and in combination with vitamin C on red blood cells (erythrocytes) counts, hemoglobin, packed cell volume (PCV%) and platelets levels in male albino rats (means±SE).

Parameter / group	Erythrocytes ($x10^{12}$/L)	Hemoglobin (g/L)	PCV (%)	Platelets ($x10^{9}$/L)
G1: Control	8.09±0.5	12.6±0.1	44.3±1.1	264.7±0.7
G2: aspirin (low)	8.3±0.07**	15±0.6**	47.8±0.98**	281±0.16
G6: Asp.(low) + vitamin	8 ±0.03##	12.3±0.8#	43.6±0.2#	299±0.2
G3 :Aspirin (high)	9.6±0.1***	18±0.3**	51±0.9**	272.8±0.9
G7 : Asp.(high) +vitamin	8.6±0.04 ###	15.9±0.2#	46.8±0.09#	311±0.8

Increase or decrease significantly relative to *the control group (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).*
Increase or decrease significantly relative to *aspirin group # $P<0.05$, ## $P<0.01$, ### $P<0.001$).*

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), superoxide dismutase (SOD) and alpha-feto protein (AFP) were calculated in control and treated groups and the results are shown in the table (4). Aspirin in low or high doses induced significant rise in AST and ALT (($p<0.05$ and $p<0.01$ respectively) relative to the control group. Co-administration of vitamin C with low and high doses of aspirin substantially ($p<0.05$) and $p<0.01$) reduced serum AST and ALT. The low dose of aspirin did not induce significant effect on MDA or SOD. Co-administration of vitamin C with the low dose of aspirin significantly ($p<0.05$) decreased MDA and increased SOD levels. The high dose of aspirin significantly ($p<0.05$) increased MDA and decreased SOD levels relative to the control group. Co-administration of vitamin C with the high dose of aspirin significantly ($p<0.05$) decreased MDA and increased SOD levels. Administration of low or high doses of aspirin showed a significant decrease ($p<0.05$) in AFP level against control group.

Table 4: Impact of low or high doses of aspirin alone and in combination with vitamin C on malondialdehyde (MDA), superoxide dismutase (SOD), Alpha-feto protein (AFP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in serum of male albino rats (means±SE).

Parameter / group	MDA (Nmol/ml)	SOD (U/ml)	AFT (u/l)	AST (u/l)	ALT (u/l)
G1 : Control	1.48±0.0	4.8±0.0	1.31±0.0	88±0.0	44±0.0
G2: Aspirin (low)	1.6±0.1	4.5±0.38	0.9±0.02*	145±13.6*	53±1.3**
G6: Asp.(low) +vitamin	1.4±0.01#	5.2±0.18#	1.1±0.01	68±6.2#	43.8±3.8##
G3: Aspirin (high)	1.8±0.4*	4.2±0.1*	0.98±0*	153±15*	63±0.75**
G7: Asp.(high) +vitamin	1.3±0.2 #	5.5±0.3#	1.09±0.01	103±12.7#	38±3.4##

Increase or decrease significantly relative to *the control group (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).*
Increase or decrease significantly relative to *aspirin group (# $P<0.05$, ## $P<0.01$, ### $P<0.001$)*

In the control and treated groups, serum creatinine, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and total protein (TP) levels were calculated and the results are shown in the table (5). Administration of low or high doses of aspirin significantly ($p<0.05$) increased serum creatinine relative to the control group. Co-administration of vitamin C with the low or high doses of aspirin significantly lowered serum levels of creatinine ($p<0.05$). On the other hand, only the high dose of aspirin induced a significant decline ($p<0.05$) in total protein levels relative to the control group.

Vitamin C co-administration with the high dose of aspirin increased serum protein levels significantly ($p<0.05$). Only the high dose of aspirin induced a significant rise in TC levels ($p<0.05$) and significant decrease ($p<0.05$) in HDL-C levels relative to the control group. Vitamin C co-administration with the high dose of aspirin significantly ($p<0.05$) decreased TC levels and increased HDL-C levels. On the other hand, aspirin in high or low doses did not induce significant effect on LDL-C levels.

Table 5:Impact of low or high doses of aspirin alone and in combination with vitamin C on serum creatinine, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and total protein (TP) levels in male albino rats (mean±SE).

Parameter / group	Creatinine (Mg/dl)	TC (Mg/d)	HDL-C (Mg/dl)	LDL-C (Mg/dl)	TP (g/dl)
G1 :Control	1±0.0	66.5±0.0	18±0.0	50.3±0.0	5.7±1.5
G2: Aspirin (low)	1.8±0.04*	67±0.6	19.5±0.3	52.8±2.1	4.9±0.2
G6: Asp.(low) +vitamin	1.2±0.17#	63.5±0.29	17.4±2.3	49±9.2	5.1±0.2
G3 Aspirin (high)	2.8±0.30*	81±0.85*	12±2.4*	64±3.9	2±0.3*
G7:Asp.(high) +vitamin	1.7±0.24#	72±0.7#	18.2±0.2#	58±3.8	2.6±0.1#

Increase or decrease significantly relative to the control group (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).
Increase or decrease significantly relative to aspirin group (# $P<0.05$, ## $P<0.01$, ### $P<0.001$)

DISCUSSION

Harmful impacts like gastrointestinal irritation, renal and hepatic toxicity, homeostasis disturbance, and reproductive problems continue, despite broad attempts to improve the safety, effectiveness, and efficacy of NSAIDs (Harris *et al*., 2002). Due to the accepted protective influences of vitamin C dietary supplementation against different pathologies, this study focused on its efficacy in countering changes caused by aspirin. Intriguingly, the findings indicate that vitamin C enhances the disordered states herein. While aspirin toxicity has been identified in several previous researches, the fundamental mechanism of toxicity is not known. Cyclo-oxygenase and thus prostaglandin biosynthesis are inhibited by NSAIDs and it has been suggested that this biochemical interference not only is the foundation of their therapeutic effects, but also leads to their side impacts (Ergul *et al*., 2010).

The humoral arm of adaptive immunity is normally correlated with circulating antibodies/immunoglobulins (Igs). T cells and antigens activate naive B cells during the primary antibody response, that then distinguish into short-lived plasma cells, long-lived plasma cells or memory B cells. First, short-lived plasma cells are produced that lead to the onset of the primary antibody response with rapid antigen-specific defense. This includes an

early increase in antigen-specific IgM class (isotype) antibodies, accompanied by maturation of affinity, isotype switching, and an increase in IgG, IgA, and/or IgE antibodies specific to the antigen (Crotty and Ahmed, 2004).

In the current study, the significant decrease in IgM production and significant increase in IgG levels after aspirin administrations indicate that aspirin can affect and regulate humoral immune response. Low IgM levels may define some humoral immunodeficiencies. On the other hand, high IgG levels may be indications of hematological disorders and/or hepatotoxic effect (Carroll and Simonson, 2005). However, aspirin did not induce any significant effects on IgA or IgE levels. Different lines of evidence suggested that aspirin can suppress the antibody-mediated humoral immune response and may weaken the host defense which include: in activated human peripheral blood mononuclear cells (PBMCs), Aspirin reduced the production of IgM and IgG antibodies; aspirin induced progressive decrease in serum IgA levels in patients with juvenile rheumatoid arthritis and inhibition of T cell-dependent antibody response in pregnant Sprague-Dawley rat juvenile males (Kushima *et al.,* 2007). In contrast, a previous study indicated that aspirin was unable to influence the primary humoral response in peripheral rabbit blood (Debowy *et al.,* 1988).

The significant changes in white blood cells in the current study representing in the significant decrease in neutrophils and lymphocytes counts and significant increase in monocytes count indicate that aspirin could affect cell-mediated immunity and that the body has been interrupted in targeting and killing invading bacteria and other harmful agents (phagocytosis). Neutrophils are the key players of innate immunity. Aspirin may inhibit the innate immune responses mediated by neutrophils by reducing their extravasation, a characteristic step in innate immunity. Various evidences suggested that aspirin by its COX-independent mechanism could minimize the adhesion of neutrophils to the endothelial lining. Another study showed that by producing the so-called aspirin-triggered resolvin D1, aspirin would suppress transendothelial neutrophil migration. Another interesting characteristic of the immunosuppressive properties of aspirin toward innate immunity is the potential to cause apoptosis in neutrophils (Sun *et al*., 2007).

In addition to sharing a protective alliance with neutrophils in the innate immunity, monocytes/macrophages also engage in regulating an adaptive-immune attack (Al-Rekabi *et al*., 2013). The present results indicated significant increase in monocyte count after aspirin intake. Supportive evidence from many lines also shows the immunopotentiating function of aspirin in innate responses mediated by monocyte/macrophage. It involves: an increase in circulating monocytes in volunteers infected with rhinovirus; rising activation of monocytes/macrophages along with upregulation of CD-11b and stimulated macrophage phagocytosis of apoptotic neutrophils in vivo (Mitchell *et al*., 2002). However, the significant increase in monocyte count in the present study possibly means that aspirin did not disrupt with the body's phagocytic function.

In the present study, the significant decrease in lymphocytes count after administration of aspirin indicated that aspirin has affected the body's acquired immune response. Previous studies indicated that by interfering with adhesion and transmigration processes, aspirin can inhibit T-cell tissue recruitment and can therefore control T-cell-mediated intravascular and extravascular adaptive immune disorders (Gerli *et al*., 2001). Aspirin may also have a direct suppressive influence on the activation or proliferation of T cells. A substantial reduction in the percentage and number of CD4+ T cells in the periphery has been shown in the therapy of BALB/c mice with low or high doses of aspirin for 4 successive weeks (Javeed *et al*., 2009). In human peripheral blood mononuclear cells (PBMCs), aspirin has also induced dose-

dependent suppression of the proliferation of T lymphocytes by halting their growth in the Go/G1 stage (Schroecksnadel *et al*., 2005). These findings indirectly suggest the immunosuppressive potential of aspirin against T cell-mediated immunity. Moreover, aspirin has been described to induce apoptosis in lymphocytes. Additional evidence suggested that through COX-independent pathways, aspirin caused apoptosis of B chronic lymphocytic leukemia cells (Bellosillo *et al.,* 1998).

The significant increasing effect of 28-days treatment of rats with the low and high doses of aspirin on RBCs counts indicated that RBCs had not been destroyed and that the rate of output of RBCs had changed (erythropoiesis). This may be explained by the finding that aspirin, the humoral regulator of the production of RBCs, has the ability to induce the release of erythropoietin from the kidneys (Polenakovic and Sikole, 1996). Therefore, aspirin therapy in rats does not have the ability to cause anemia. Aspirin treatment in rats may also have a potential effect on bone marrow, kidney and hemoglobin metabolism, because only materials which have a major impact on red blood cell values and related parameters have been reported to influence bone marrow, kidney and hemoglobin metabolism (Young and Maciejewski, 1997). The significant increases in PCV% and Hb values caused by low and high doses of aspirin in the present study could indicate an improvement in the blood's oxygen carrying capacity and the amount of oxygen delivered to the tissues and that aspirin could protect against anemia. On the other hand, previous studies showed that aspirin can have a harmful impact on the blood chemistry of albino rats (Li *et al.*, 2012).

The rise in ALT and AST serum levels is deemed to be an early step in the identification of liver damage (Kaneko, 1985). In this study, aspirin-induced liver toxicity is obvious and the substantial rise in AST and ALT levels noted in aspirin treated rats is consistent with several studies in experimental animals (Yousef *et al.,* 1999) who proved that non-steroidal anti-inflammatory medicines like aspirin, ibuprofen and naproxen can modify liver function and cause serum AST and ALT elevation and hepatic cell necrosis, as hepatic damage triggers enzyme leakage from the cells due to changes in membrane permeability, resulting from increased secretory activity and disruption of hepatocyte transport function (Fan *et al.,* 2009).

The present study showed that aspirin in the high dose only caused significant increase in the MDA level and a significant decline in the SOD level relative to the control group. This indicates that aspirin may induce marked disturbances in antioxidant defense system and oxidative stress since MDA, that is the last product in the process of lipid peroxidation, is deemed a marker of oxidative stress (Ayala *et al.,* 2014). A significant indicator of kidney function is a change in serum creatinine levels. The significant elevations in the levels of serum creatinine when compared to the normal rats in the current study indicate that this drug may adversely affect renal function. The renal dysfunction linked to glomerular and tubular degeneration induced by aspirin toxicity may explain these impacts. Negative impacts after administration of aspirin to normal rats can be the result of ANSIDs induced inhibition of prostaglandin synthesis that lead to renal vasoconstriction, and reduced renal perfusion that is accountable for acute renal disorders (Matzke, 1996).

The decrease in total protein noticed in the present study after aspirin administration is in line with other results which indicated decreased levels of total protein caused by NSAID intoxication due to disruption of lysosomal membranes under the influence of NSAID

poisoning, resulting in the release of their hydrolytic enzymes into the cytoplasm, resulting in marked lysis and dissolution of the target material (Ebaid *et al.,* 2007). Moreover, decreased levels of serum protein may be due to decreased synthesis of hepatic DNA and RNA that in turn influences the use of free amino acids to synthesize protein (Aprioku *et al.,* 2014).

An elevated risk of atherosclerosis, cardiovascular disease and stroke is linked to high blood cholesterol levels (Chatterjea *et al.,* 2007). Administration of aspirin in the low dose in the present study did not significantly affect the total cholesterol, HDL-C and LDL-C levels. While, high-dose aspirin administration induced higher levels of total cholesterol and decreased levels of HDL-C, which may be a possible indicator of disruption of fatty acid metabolism, and indirectly of potential membrane lipid peroxidation and free radical release affected by exposure to aspirin (Ergul *et al.,* 2010). Alpha-fetoprotein (AFP) is a type of protein found in adults and produced in the developing fetus. Its serum concentration can be used to confirm hepatocarcinoma and for the diagnosis of tumor. If a person has high blood levels of AFP, it can be an indicator of liver cancer (Wong *et al.,* 2013). In the present study, aspirin could not increase the level of AFP significantly relative to the control group. The findings of the current research support the safety of aspirin and indicate that this drug has not carcinogenic effects.

The possible ameliorating effect of vitamin C on the previous parameters may be due to its antioxidant effect. Furthermore, ascorbic acid is a powerful scavenger of plasma reactive oxygen species and extracellular liver compartments (Inoue, 1994 and Ergul *et al.,* 2010). One of the most essential vitamin C functions is to support and energize the immune system of the body. In the current study, the significant increasing effect on WBCs, neutrophils and lymphocytes levels after co-administration of vitamin C with aspirin indicates that vitamin C can activate the immune response. Previous studies showed that vitamin C supports many aspects of the neutrophil functions, moreover, intake of antioxidant vitamins was proven to result in a significant rise in lympho-proliferative ability and in phagocytic functions of polymorphonuclear neutrophils (Anderson, 1981). The immuno-enhancer effect of vitamin C was demonstrated in the current study by the significant increase in IgM levels. These results are in accordance with previous studies that showed that ascorbic acid raises the levels of IgM, as well as, the concentration of complement (C3) in the bloodstream and that increased levels of IgM were found in the women who supplemented their diet with vitamin C (Acid, 1977). On the other hand, serum IgG level was reduced significantly in the current study after supplementation with vitamin C, suggesting that the downregulating effects on immunoglobulins can reveal beneficial effects of vitamin C in hypersensitivity and autoimmunity. The improving effects of vitamin C on RBCs parameters could be attributable to the capacity of this antioxidant vitamin to control and enhance iron absorption from the gut by controlling the reduction of oxidized iron to its reduced form (Pavlovic and Sarac, 2011). However, vitamin C could induce significant effects on the different hematological parameters that may be due to ameliorating the disturbed bone marrow activity.

CONCLUSION:

We could conclude that aspirin caused immunological and hematological alterations, in addition to hepatotoxic and nephrotoxic effects in male albino rats at the used doses and duration and vitamin C supplementation, might counteract the toxic impacts of aspirin. Aspirin affected both cell-mediated and humoral immunity and vitamin C could exert immunomodulatory action. Moreover, the two doses of aspirin did not exhibit carcinogenic or

anemic effects and the adverse effects of aspirin were dose-dependent as the low dose had less adverse effects than the high dose.

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