Journal of Nutrition and Health Sciences
Volume 5 | Issue 4
ISSN: 2393-9060

Review Article Open Access

Beneficial Effects of Vitamin E against Indomethacin-induced Glutathione Depletion, Acetylcholinesterase Activity, (Na^+,K^+)-ATPase Activity and Osmotic Fragility of Erythrocyte

Ahmad I[#1], Swaroop A[2] and Bagchi D[*2,3]

[1]Cepham Life Sciences, Inc., Baltimore, MD 21090, USA
[2]Cepham Inc., Somerset, NJ 08773, USA
[3]Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX 77002, USA
[#]The experiments were conducted by Ahmad I in the Department of Biochemistry, University of Allahabad, India

[*]**Corresponding author:** Bagchi D, PhD, MACN, CNS, MAIChE, Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX 77002, USA, E-mail: debasisbagchi@gmail.com

Citation: Ahmad I, Swaroop A, Bagchi D (2018) Beneficial Effects of Vitamin E against Indomethacin-induced Glutathione Depletion, Acetylcholinesterase Activity, (Na^+,K^+)-ATPase Activity and Osmotic Fragility of Erythrocyte. J Nutr Health Sci 5(4): 408

Received Date: July 12, 2018 **Accepted Date:** December 29, 2018 **Published Date:** December 31, 2018

Abstract

Background: Indomethacin, is a well-known non-steroidal anti-inflammatory drug (NSAID), a cyclooxygenase inhibitor and extensively used as a medicine to reduce pain, fever, stiffness, swelling or inflammatory responses. Indomethacin inhibits both COX-1 and COX-2, which in turn, inhibits the production of prostaglandins in the gastrointestinal tract and disrupts the mucosal lining in the gastrointestinal tract leading to intestinal bleeding and perforation.

Methods: The protective ability of α-tocopheryl acetate (Vitamin E, Vit E) was assessed against indomethacin-induced glutathione depletion, increase in acetylcholinesterase (AChE), (Na^+,K^+)-ATPase activities and decrease in erythrocyte osmotic fragility in the red blood cells sample isolated from male albino rats.

Results: Indomethacin significantly inhibited reduced glutathione (GSH), which was dramatically protected by Vit E. However, no detrimental activities of indomethacin were observed on glutathione reductase (GR) or glutathione peroxidase (GPx). A significant increase in the of AChE activity of red blood cells was observed following treatment with indomethacin, however, Vit E treatment didn't bring the AChE activity to normal and further studies are needed. A small, but non-significant, elevation in the (Na^+,K^+)-ATPase activity was observed following treatment with indomethacin and slight non-significant reduction was observed following treatment with Vit E. Similar results were observed in erythrocyte osmotic fragility experiment demonstrating % hemolysis in these red blood cells.

Conclusion: Thus, Vit E may serve as an adjunct preventative agent during the therapeutic treatment with indomethacin.

Keywords: Indomethacin; Vitamin E; Tocopherol; α-Tocopherol; Glutathione; (Na^+,K^+)-ATPase Activity; Acetylcholinesterase; AChE; Erythrocyte Osmotic Fragility; Red Cell Membrane

Introduction

Overdose of indomethacin, a well-recognized nonsteroidal anti-inflammatory drug (NSAID), has been demonstrated to induce gastric mucosal injury leading to abdominal pain, bleeding, black tarry stools, weakness, dizziness, ulceration, orthostatic hypotension and perforation in the gastrointestinal tissues. Furthermore, indomethacin reduces the ability of blood to clot, restricts the blood flow to the kidneys thereby cause nephrological dysfunctions and congestive heart failure [1-5].

It has been demonstrated that a deficiency of endogenous prostaglandins due to inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) by indomethacin is involved in gastrointestinal mucosal injury, macrovascular injury, ulcer leading to gastrointestinal mucosal injury and gastric bleeding [3,5-7]. A significant number of individuals are allergic to NSAIDs and in turn, develop shortness of breath, while asthma patients have higher risk of serious allergic reactions to NSAIDs [3,5].

In this study, we focused to determine the effect of indomethacin on glutathione depletion, acetylcholinesterase (AChE) and (Na^+,K^+)-ATPase activities, and to evaluate the efficacy of vitamin E in protecting against indomethacin-induced cellular injury.

We also determined the efficacy of vitamin E in indomethacin-induced toxicity on red blood cells.

Vitamin E, a well-established fat soluble antioxidant and a member of tocopherols and tocotrienol family, intervenes the propagation of free radicals and reactive oxygen species that spread through biological membranes or lipid peroxidation [8-10]. In short, vitamin E provides marvelous protection against oxidative stress in both in vitro and in vivo models. The deactivation of protein kinase C is mediated by vitamin E to intervene smooth muscle growth [11,12]. Vitamin E has been demonstrated to downregulate the expression of the CD36 scavenger receptor gene, which in turn modulate the expression of the connective tissue growth factor [13,14].

Normal human red blood cells have been exhibited to exert a low basal permeability to the cations, which is highly temperature dependent. This effect is constantly corrected by (Na^+,K^+)-ATPase [15], while diverse stimulatory conditions, including oxidative burst or chemical insult, aggravate the incidence of cation leaks from red blood cells. The protective efficacy of vitamin E against indomethacin-induced pathophysiology was assessed.

To determine the erythrocyte resistance to hemolysis, it is a common practice to conduct osmotic fragility test. Basically, this test diagnoses the involvement of (a) thalassemia and/or (b) hereditary spherocytosis [16]. Overall, this experiment will evaluate whether following treatment with indomethacin, the characteristics of hemoglobin has been compromised. Red blood cells carry oxygen with the help of hemoglobin and performs diverse biochemical and pathophysiological functions, and any perturbation or malfunctions lead to anemia [17,18].

This study will assess whether indomethacin compromises the ability of hemoglobin. As it is known that hemoglobin assists red blood cells to carry oxygen and perform the routine biochemical and pathophysiological functions, and any disruption in this pathogenesis will cause anemia. This study will conduct erythrocyte osmotic fragility (% hemolysis) following treatment with indomethacin and determine possible protective ability of vitamin E.

This investigation is extremely important as indomethacin is extensively used worldwide and often time consumed in high doses by the patients, so this study will evaluate whether co-administration of vitamin E may provide some protection against indomethacin-induced gastrointestinal injury.

Materials and Methods

Chemicals and Reagents

All reagents were of the highest purity available. Reduced glutathione (GSH), Glutathione reductase, NADPH, 5, 5'-dithiobis-(2-nitrobenzoic) acid (DTNB), Adenosine triphosphate disodium (ATP-Na_2), Trizma (Tris-HCl) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Imidazole was purchased from Fluka AG Buchs, Switzerland and Ouabain from E. Merck, Germany. Indomethacin was obtained from Bayer-India Ltd (Thane, Maharashtra, India).

Experimental Animals and Treatment

The study protocol was approved by the Institutional Animal Ethics Committee and performed in accordance with the rules and guidelines on animal experimentation. Albino rats, *Charles foster* strain were obtained from the National Laboratory Animal Center (NLAC) of the CSIR- Central Drug Research Institute, Lucknow, UP, India, for breeding and experimental purposes. The animals were maintained on standard hygienic conditions, in the form of an open system of husbandry under natural photoperiod and the average temperature of the animal house was maintained 24±3 °C throughout the year. Young male albino rats (*Charles foster*), weighing 200-250 g, age: 60 days, maintained on commercial rat diet (Lipton Co., India Ltd./Unilever, Mumbai, Maharashtra, India), under standard hygienic conditions, were divided into three groups- Group I, Group II and Group III. The rats of Group I served as Control were given the diet and water *ad libitum*. The rats of Group II were administered non-therapeutic dose of 0.42 mg indomethacin/kg body weight *p.o*. daily. The Group III rats were administered indomethacin at a dose similar to Group II along with Vitamin E (α-tocopheryl acetate) at a dose of 6.7 mg/kg body weight/day. Since, the significant changes were observed in the case of rats treated for five days, accordingly the rats treated for five days were selected for the experiments.

Blood samples from Group I, Group II and Group III rats were collected from the caudal vein [19] after five days of continuous treatments on the sixth day. The osmotic fragility (O.F.) was determined [20] and O.F., profiles were obtained by plotting percent (%) hemolysis against the corresponding NaCl concentration (%) in the range of 0.1 to 0.9 %.

Estimation of Reduced Glutathione (GSH)

The method of Beutler *et al.* [14] was adapted for erythrocyte reduced glutathione (GSH) estimation, using 5, 5'-dithiobis-(2-nitrobenzoic) acid as the reaction agent. The GSH standard 2 µg-100 µg demonstrated the absorbance (412 nm) linear relationship with reduced glutathione concentration.

Glutathione Reductase (GR) and Glutathione Peroxidase (GPx)

The red cell enzyme activities of GR and GPx were assayed as per the method of Beutler *et al.* [21]. The activity of GR enzyme was

measured following the oxidation of NADPH at 340 nm. The final assay mixture contained 50mM Tris-HCl, 0.25 mM EDTA (pH 8.0), 1 µM FAD, 3 mM GSSG, 0.1 mM NADPH and 30 µl 1:20 hemolysate.

The activity of the enzyme GPx was assayed by measuring the oxidation of GSH to GSSG by t-butylhydroperoxide, then measuring its rate of formation, using the GR reaction and the oxidation of NADPH was followed at 340 nm. The final assay mixture contained 100 mM Tris-HCl, 0.5 mM EDTA (pH 8.0), 2 mM GSH, 0.3 mL of 10 units/mL of GR, 0.2 mM NADPH, 0.07 mM t-butylhydroperoxide, and 30 µL of 1:20 hemolysate.

Acetylcholinesterase (AChE) Activity

AChE activity was determined following the method of Beutler *et al.* [22]. In the assay system, acetylthiocholineiodide was used as the substrate. The rate of production of thiocholineiodide by AChE was measured, using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which produced yellow color and its rate of formation was recorded at 412 nm. The final assay mixture contained 100 mM Tris-HCl, 0.5 mM EDTA (pH 8.0), 0.025 mM DTNB in 0.1% Sodium citrate and 50 µl of 1:20 diluted hemolysate.

Preparation of Red Cell Membrane and Assay of (Na^+,K^+)-ATPase Activity

The blood samples were centrifuged for 15 minutes at 3500 rpm, the plasma and buffy coats were removed and the sedimented red cells were washed 4-5 times with 0.154 M (physiological concentration) of NaCl. Precaution was taken to eliminate leukocyte contamination. The red cell membranes were prepared following the method of Marchesi and Palade [23] and the entire procedure was carried out at 4 °C. The activity of the enzyme (Na^+,K^+)-ATPase was assayed following the method of Lukacovic *et al.* [24].

Hemoglobin (Hb) and Erythrocyte Osmotic Fragility (O.F., % Hemolysis) Assay

Hb estimation was performed following the method of Beutler *et al.* [21,22], while erythrocyte osmotic fragility (% hemolysis) tests were performed following the method of Dacie and Lewis [20]. For hemoglobin estimation, 20 µl of hemolysate was added in 1 mL Drabkin's Solution (Ferricynide-cyanide reagent) and the OD was measured at 540 nm within 5 minutes. For O.F. measurements, heparinized blood was added to hypotonic solutions of varying concentration of NaCl in the range of 0.1% to 0.9% and the tubes were allowed to stand at room temperature for 30 minutes. The tubes were then centrifuged in a clinical centrifuge at 2000 rpm for 5 minutes and optical density of the supernatant was measured at 440 nm, using supernatant form 0.9% NaCl as blank, having undetectable lysis.

Statistical Analysis

The results are expressed as means ± SD. All the data of different treated groups as compared to the control groups were statistically analyzed, using Student's t-test and values $p<0.05$ were considered significant.

Results

Reduced Glutathione (GSH), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx)

Significant reduction was observed in GSH level following treatment with Indomethacin (0.42 mg/kg body weight) over a period of five days, however, vitamin E (6.7mg/kg body weight) provided significant protection ($p <0.001$). Incidentally, co-treatment of the red blood cells with indomethacin and vitamin E provided greater than 90% protection. However, no significant changes were observed in glutathione reductase (GR) or glutathione peroxidase (GPx) levels following treatment of the red blood cell sample with indomethacin or indomethacin + Vitamin E (Table 1).

Rat Blood Cell Sample	Reduced Glutathione (GSH)*		Glutathione Reductase (GR)*[b]	Glutathione Peroxidase (GPx)*[b]
	mg/100mL RBC	mg/g Hb	IU/g of Hb	IU/g of Hb
Control	24.42 ±1.18	1.58 ±0.121	7.34 ±0.34	25.23 ±1.02
Indomethacin	20.44 ±0.90[a]	1.426 ±0.007[a]	7.38 ±0.39	25.90 ±0.91
Indomethacin + Vitamin E	23.54 ±0.30[a]	1.487 ±0.045	7.85 ±0.40	24.95 ±1.21

* Each value is the mean of at least 3-4 experiments ±SD
a $P< 0.001$; highly significant when compared with control
b Enzyme activity is expressed in terms of IU (µmole of NADPH oxidized/minute) per g of hemoglobin at 37 °C
Table 1: Reduced glutathione (GSH), Glutathione reductase (GR) and Glutathione peroxidase (GPx) levels in Red Blood Sample of Control, Indomethacin-treated and Indomethacin with Vitamin E-Treated Rats

Acetylcholinesterase (AChE) And (Na^+,K^+)-ATPase Activities In Red Blood Cells

Indomethacin (0.42 mg/kg body weight)-induced a significant increase in AChE activity, while indomethacin and vitamin E co-treatment caused no reversal of this effect (Table 2). A slight, but non-significant, increase in (Na^+,K^+)-ATPase activity was observed following treatment with indomethacin (0.42 mg/kg body weight) while indomethacin and vitamin E co-treatment exhibited a marginal decline (Table 2).

Rat Blood Cell Sample	Acetylcholinesterase (AChE) Activity*[b]	(Na^+,K^+)-ATPase Activity*[c]
	IU/g of Hb	µmole Pi/mg Protein
Control	16.55 ±1.27	0.162 ±0.010
Indomethacin	29.42 ±0.96[a]	0.168 ±0.008
Indomethacin + Vitamin E	27.06 ±1.71	0.166 ±0.006

* Each value is the mean of at least 3-4 experiments ±SD
a P< 0.001; highly significant when compared with control
b Enzyme activity is expressed in terms of IU (µmole of acetyl thiocholine iodide hydrolyzed/minute) per g of hemoglobin at 37 °C
c Enzyme activity is expressed in terms of µmole Pi liberated per hour per mg of protein.
Table 2: Red cell membrane Acetylcholinesterase (AChE) and (Na^+,K^+)-ATPase Activities of Control, Indomethacin treated and Indomethacin with Vitamin E Treated Rats

Erythrocyte Osmotic Fragility (%Hemolysis of the Red Blood Cells)

Indomethacin-induced erythrocyte osmotic fragility or percentage of hemolysis of the red blood cells was determined to assess the protective ability of vitamin E. Erythrocyte osmotic fragility (% Hemolysis) of control, indomethacin-treated and indomethacin + vitamin E-treated rats are shown in (Table 3). Indomethacin treatment caused no significant changes in erythrocyte osmotic fragility, when compared with control, and vitamin E treated samples.

	% Hemolysis		
% NaCl	**Control (No Drug Treatment)**	**Indomethacin**	**Indomethacin + Vitamin E**
0.1	98.91 ±0.78	98.29 ±1.38	98.74 ±0.70
0.2	97.23 ±2.23	94.16 ±1.59	94.53 ±0.52
0.3	100 ±0.00	100 ±0.00	100 ±0.00
0.4	97.64 ±2.05	97.83 ±0.96	96.89 ±0.80
0.5	96.27 ±1.16	96.18 ±2.04	94.98 ±1.77
0.6	92.2 ±1.64	92.69±2.84	92.84 ±2.68
0.65	92.27 ±3.54	85.86 ±3.17	86.96 ±3.59
0.7	76.39 ±3.94	59.49 ±2.59	66.07 ±15.19
0.75	43.80 ±7.87	24.05 ±3.88	36.29 ±9.00
0.8	14.22 ±4.98	6.78±0.77	8.65 ±1.36
0.85	4.34 ±0.97	1.43 ±0.96	2.79 ±0.47
0.9	0.00 ±0.00	0.00 ±0.00	0.0 0.00

Erythrocyte osmotic fragility was assessed as a % Hemolysis of control, Indomethacin-treated and Indomethacin + vitamin E-treated rats. % Hemolysis was assessed at various concentrations of NaCl. See Materials and Methods section for details.
Table 3: Erythrocyte Osmotic Fragility (% Hemolysis) of Control, Indomethacin treated and Indomethacin with Vitamin E Treated Rats

Discussion

Indomethacin is a well-established NSAID and widely used as an effective anti-pyretic, analgesic and anti-inflammatory drug. Structurally, it is a methylated indole derivative having analgesic-antipyretic properties similar to other analgesic antipyretic drugs. Indomethacin inhibits prostaglandin synthesis by inhibiting prostaglandin-forming cyclooxygenase and is one of the most potent inhibitors of cyclooxygenase [25,26]. After oral administration, indomethacin is absorbed rapidly and almost completely from the gastrointestinal tract. The peak plasma concentration reaches within 3 hours after administration and ninety percent of the indomethacin becomes bound to plasma protein. Indomethacin is metabolized in liver by hepatic microsomal enzymes and has been reported to be largely converted to inactive metabolites [27,28].

In the 1960s, a number of acetic acid derivatives were developed into indomethacin, diclofenac, and sulindac, and propionic derivatives into ibuprofen, naproxen, and ketoprofen [29]. Indomethacin exhibited the most potent activity of all these derivatives. Since then, indomethacin is used in treatment of migraine, headaches and pain alleviation, and became renowned as a headache reliever, or more so, as "indomethacin-responsive" headache disorders [28,29].

Following oral administration, indomethacin is rapidly absorbed in the gastrointestinal tract and reported to be virtually 100% bioavailable with peak plasma concentrations after oral administration of a single dose, between 0.9 + 0.4 and 1.5 + 0.8 hours in an empty stomach [26,27]. However, indomethacin bioavailability and absorption is significantly reduced and delayed when taken with meal, while the maximal effect has been reported when taken after a high protein diet, or high-lipid diet. Furthermore, it is interesting to note that advancing age doesn't affect indomethacin absorption or bioavailability [26,27].

It has been repeatedly demonstrated that acute renal failure caused by the NSAIDs administered at therapeutic doses is an underestimated complication as it is usually mild, transitory and non-anuric [26,27,30]. Literature exhibits that NSAIDs including

chronic and overdose use of indomethacin causes renal toxicity by inhibiting the enzyme cyclooxygenase in the glomerulus, producing vasoconstriction. Especially in premature neonates, it has been estimated that therapeutic doses of indomethacin give rise to transitory renal toxicity in 24% of cases [26,27]. Moreover, there is a higher risk of drug-induced kidney toxicity and failure in critically ill patients due to their pre-existing renal complications including renal failure, presence of secondary hypovolemia, hypertension, cardiac failure, hypoalbuminemia, electrolyte disturbances, hepatic disorders affecting metabolism, and co-administration of other prescribed drugs may interfere with the metabolism and/or potentiate the nephrotoxicity [26,27,30].

Since, indomethacin is extensively used popular drug and is associated with adverse reactions and toxicity; we undertook this project to find out whether vitamin E can provide protection against indomethacin-induced toxicity. We demonstrated the effect of indomethacin on glutathione depletion, acetylcholinesterase (AChE) and (Na^+,K^+)-ATPase activities in red blood cells and evaluated the efficacy of vitamin E in protecting against indomethacin-induced cellular injury. We also determined the efficacy of vitamin E in indomethacin-induced erythrocyte osmotic fragility. Earlier, we studied the effect of another NSAID, acetylsalicylic acid on red cell osmotic fragility, reduced glutathione level, AChE and (Na^+,K^+)-ATPase activities [31].

Our study demonstrated a significant reduction in GSH level following treatment with indomethacin, however, vitamin E treatment provided a significant protection ($p<0.001$) in GSH level, but no changes were observed in glutathione reductase (GR) or glutathione peroxidase (GPx) levels. Indomethacin (0.42 mg/kg body weight)-induced a significant increase in AChE activity, while indomethacin and vitamin E co-treatment caused no reversal of this effect. We need to do further investigation to unveil this mechanism of action.

A small, but non-significant, increase in (Na^+,K^+)-ATPase activity was observed following treatment with indomethacin, while indomethacin and vitamin E co-treatment exhibited a marginal decline. Indomethacin treatment caused a significant decrease in erythrocyte osmotic fragility, when compared with control, while vitamin E showed protection.

Taken together, this investigation exhibits that vitamin E may serve as a novel adjunct therapy in conjunction with indomethacin therapy.

References

1. Summ O, Evers S (2013) Mechanism of action of indomethacin-responsive headaches. Curr Pain Headache Rep 17: 327-32.

2. Mitra S, Florez D, Tamayo ME, Mbuagbaw L, Vannivasingam T, et al. (2018) Association of Placebo, Indomethacin, Ibuprofen, and Acetaminophen With Closure of Hemodynamically Significant Patent Ductus Arteriosus in Preterm Infants: A Systematic Reviewand Meta-analysis. JAMA 319: 1221-38.

3. Simon LS (1993) Nonsteroidal anti-inflammatory drug toxicity. Curr Opin Rheumatol 5: 265-75.

4. Naito Y, Yoshikawa T, Yoshida N, Kondo M (1998) Role of oxygen radical and lipid peroxidation in indomethacin-induced gastric mucosal injury. Dig Dis Sci 43: 30S-4S.

5. Blumenthal KG, Lai KH, Huang M, Wallace ZS, Wickner PG (2017) Adverse and Hypersensitivity Reactions to Prescription Nonsteroidal Anti-Inflammatory Agents in a Large Health Care System. J Allergy Clin Immunol Pract 5: 737-43.

6. Drugs and Supplements (2018) Indomethacin (oral route): side effects.

7. WebMD (2018) Indomethacin Side Effects by Likelihood and Severity.

8. Abraham A, Kattoor AJ, Saldeen T, Mehta JL (2018) Vitamin E and its anticancer effects. Crit Rev Food Sci Nutr 10: 1-8.

9. Reboul E (2017) Vitamin E bioavailability: mechanism of intestinal absorption in the spotlight. Antioxidants (Basel) 6: E95.

10. Comitato R, Ambra R, Virgili F (2017) Tocotrienols: afamily of molecules with specific biological activities. Antioxidants (Basel) 6: E93.

11. Stocker A (2004) Molecular mechanisms of vitamin E transport. Ann N Y Acad Sci 1031: 44-59.

12. Glauert HP (2007) Vitamin E and NF-kappaB activation: a review. Vitam Horm 76: 135-53.

13. Pruthi S, Allison TG, Hensrud DD (2001) Vitamin E supplementation in the prevention of coronary heart disease. Mayo Clin Proc 76: 1131-6.

14. Kaempf-Rotzoll DE, Traber MG, Arai H (2003) Vitamin E and transfer proteins. Curr Opin Lipidol 14: 249-54.

15. Flatt JF, Bruce LJ (2018) The Molecular Basis for Altered Cation Permeability in Hereditary Stomatocytic Human Red Blood Cells. Front Physiol 9: 367.

16. Emilse LAM, Cecilia H, Maria TM, Eugenia MM, Alicia IB, et al. (2018) Cryohemolysis, erythrocyte osmotic fragility, and supplementary hematimetric indices in the diagnosis of hereditary spherocytosis. Blood Res 53: 10-7.

17. Ford J (2013) Red blood cell morphology. Int J Lab Hematol 35: 351-7.

18. Segel GB, Lichtman MA (2014) Direct antiglobulin ("Coombs") test-negative autoimmune hemolytic anemia: a review. Blood Cells Mol Dis 52: 152-60.

19. Omaye ST, Skala JH, Gretz MD, Schaus EE, Wade CE (1987) Simple method for bleeding the unanaesthetized rat by tail venipuncture. Lab Anim 21: 261-4.

20. Dacie JV, Lewis SM (1984) Practical Hematology (6th Edn) Churchill Livingstone, Edinburgh, Scotland.

21. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61: 682-8.

22. Beutler E (1984) Glutathione peroxidase (GSH-Px) In: Red cell metabolism: A manual of biochemical methods. Grune & Stratton, New York 1984: 72-6.

23. Marchesi VT, Palade GE (1967) The localization of Mg-Na-K-activated adenosine triphosphate on red cell ghost membranes. J Cell Biol 35: 385-404.

24. Lukacovic MF, Toon MR, Solomon AM (1984) Site of red cell cation leak induced by mercurial sulfhydryl reagents. Biochim Biophys Acta 772: 313-20.

25. Goodman L, Gilman LA (1985) The Pharmacological Basis of Therapeutics (7th Edn) Macmillan Publishing Co, USA.

26. Lucas S (2016) The pharmacology of indomethacin. Headache 56: 436-46.

27. Duggan DE, Hogans AF, Kwan KC, McMahon FG (1972) The metabolism of indomethacin in man. J Pharmacol Exp Ther 181: 563-75.

28. VanderPluym J (2015) Indomethacon-responsive headaches. Curr Neurol Neusosci Rep 15: 516.

29. Shen TY, Winter CA (1977) Chemical and biological studies on indomethacin, sulindac and their analogs. Adv Drug Res 12: 90-245.

30. Aranda JV, Thomas R (2006) Systematic review: intravenous ibuprofen in preterm newborns. Semin Perinatol 30: 114-20.

31. Ahmad I, Swaroop A, Bagchi D (2018) Protective ability of vitamin E against acetylsalicylic acid-induced glutathione depletion, acetylcholinesterase and (Na+,K+)-ATPase activities and erythrocyte osmotic fragility. J Nutr Health Sci 5: 308.