Evaluation of Ibuprofen acid effect on oxidative stressed mice

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ABSTRACT

Ibuprofen ((RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid) is a well-known non-steroidal anti-inflammatory drug (NSAIDs) widely used for the treatment of pain, fever and inflammation. This study designed to evaluate if dose of 30mg/kg of Ibuprofen has an antioxidant effect, relative to its analgesic, antipyretic and anti-inflammatory activities. Oxidative stress was induced by intraperetoneal injection of (100 mg/kg) peroxide hydrogen (H_2O_2), and then a comparative study is made concerning the activities of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), gluthation reductase (GR), succinate deshydrogenase (SDH), and index of lipid peroxidation: thiobarbituriquc acid reactive substances (TBARS) determined lipid peroxidation in mice treated with H_2O_2 accompanied by Ibuprofen; compared to the group treated by L-ascorbic acid + H_2O_2, These results suggest that Ibuprofen may exert protective effects against oxidative stress damages.

Keywords: Ibuprofen, oxidative stress, antioxidant, anti-inflammatory, peroxide hydrogen (H_2O_2).
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INTRODUCTION

Ibuprofen was introduced as a non-steroidal anti-inflammatory drug (NSAID) in the United Kingdom in 1969 and in the United States in 1974. It was developed directly as a result of the problems associated with the use of corticosteroids in the treatment of rheumatoid arthritis and also because of the gastro-intestinal irritation and general intolerability of the NSAIDs available at that time [1 and 2]. Ibuprofen is a chiral NSAID of the 2 arylpropionic acid class, chemically related to fenoprofen and naproxen, has moderate but definite anti-inflammatory properties, with considerably less gastrointestinal adverse effect than other NSAIDs [3]. The drug has analgesic and antipyretic properties, probably related to its anti-inflammatory effects [4]. Ibuprofen is effective not only in mild inflammation but also in severe inflammatory diseases such as rheumatoid arthritis and osteoarthritis and is probably effective in ankylosing spondylitis, gout, and Bartter's syndrome [1 and 2]. Substantial concentrations of ibuprofen are indeed attained in synovial fluid, which is a proposed site of action of its NSAID activity [1 and 2].

Therefore, new NSAIDs without these side effects have long been pursued. The major mechanism by which ibuprofen and other NSAIDs exert their anti-inflammatory activity is through the suppression of prostaglandin biosynthesis by inhibiting cyclooxygenases (COXs). Prostaglandins are major mediators of inflammation response, but also play a cytoprotective role in maintaining GI health and homeostasis. COXs, which catalyze the synthesis of prostaglantins from arachidonic acid, have two major subtypes COX-1 and COX-2 [5].

Oxidative stress takes place when the balance between the antioxidant defenses and the generation of reactive oxygen species (ROS) is tipped in favor of the latter. Thus, hydrogen peroxide (H2O2) is directly involved in the production of ROS due to his high redox level. if not maintained oxidative damage accumulates known as oxidative

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stress [6]. ROS are products of regular cell metabolism ($^1O_2$: singlet oxygen, H_2O_2: hydrogen peroxide, (OH$^•$)**:** hydroxyl radical, (O2$^{•-}$): anion superoxide). They participate in many cellular events including signal transduction and antibacterial defense [7]. They are also capable a large dose of oxidizing cellular proteins, nucleic acids and lipids [8]. They contribute to cellular aging [9], mutagenesis [10] carcinogenesis [11] Alzheimer's disease [12], atherosclerosis (13) and coronary heart disease [14] possibly through destabilization of membranes [15], DNA damage [16], and oxidation of low-density lipoprotein [17].

To protect from these highly reactive intermediates, living organisms possess a defense system consisting of enzymatic and non-enzymatic antioxidants that scavenge them. It is well established that the most important antioxidant enzymes are superoxide dismutase (SOD), which ensures the dismutation of superoxide (O2$^{•-}$) into a molecule of O_2 , catalase (CAT) which catalyzes the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen, glutathione peroxidase (GPX), which reduces both H_2O_2 and organic peroxides by a glutathione-dependent reaction, and glutathione reductase (GR), which catalyzes the NADPH dependent regeneration of glutathione (GSH) from the oxidized form (GSSG) generated by GPX. SDH catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol, this occurs in the inner mitochondrial membrane by coupling the two reactions together [18; 19; 20; 21; 22; 23; 24].

The objective of the present research was to examine and test precisely antioxidant activity of NSAID Ibuprofen and even the protective effect at long-term of treatment in liver of mice, compared and analyzed the activity of increasing stress markers that suits hydrogen peroxide by oxidation, and reduction of the activity of stress markers suitable for treatment by L-ascorbic acid, with the decrease of the activity markers stress in the groups treated by Ibuprofen.

EXPERIMENTAL SECTION

Tests:
The test concerned 66 males adult Swiss albino mice weighting 25-30 grams. They were acclimatized to laboratory conditions before the test and fed *ad libitum*. They were fasted 16 hours prior to the treatment [9].

All experiments were in accordance with the guidelines provided by the CPCSEA.

Animals were divided into 11 groups (n = 6 per group) as it's resumed in table 1. Ibuprofen, vitamin C (L-ascorbic acid) and H_2O_2 were daily administered by intraperitoneal injection during 30 days.

Table 1: summary of groups treated with Ibuprofen, H_2O_2 and Vitamin C

Groups	Number of mice	Treatment	Dose
1	6	NaCl	0.9 %
2	6	H_2O_2	100 mg/kg
3	6	Ibuprofen	30 mg/kg
4	6	Ibuprofen + H_2O_2	30 mg/kg + 1,5 g/kg
5	6	Vitamin C	20 mg/kg
6	6	Vitamin C + H_2O_2	20 mg/kg + 1,5 g/kg

Preparation of tissues for analytic procedures
Livers were rapidly thawed and homogenized using a Potter homogenizer (Elvehjem), in 3 volumes of ice-cold 10 mM HEPES, 1 mM EDTA, 0.25 M Sucrose and 10 mM 2-mercaptoethanol, pH 7.4. All procedures were performed at 4°C. Homogenates were centrifuged at 7000 x g for 15 mn at 4°C (sigma 2-16K) and the resultant supernatants were aliquoted and stored at – 20°C for later enzyme assays.

Biochemical assays
All assays were conducted at 25°C using Jenway 6405 UV/Visible spectrophotometer (Thermo electron corporation, Biomate 3).

Protein Assay
Protein content was measured according to the Bradford procedure [10] by using bovine serum albumin (BSA) as standard. Protein reagent was added to protein solutions. The absorbance was measured at 595 nm after 10-15 mn of incubation in the dark.

Catalase
The consumption of 7.5 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7) was monitored at 240 nm as indicated in [11].

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Glutathione reductase
The assay of Di ilio et al., (1983) [12] was used. The assay mixture contained 0.5 mM oxidized glutathione, 1 mM EDTA, 0.1 mM NADPH and 50 mM potassium phosphate buffer (pH 7.4) and NADPH consumption was monitored at 340 nm.

Superoxide dismutase
The enzyme was assayed according to Paoletti et al., (1986) [13]: 5 mM EDTA, 2.5 mM $MnCl_2$, 0.27mM NADH, 3.9 mM 2-mercaptoethanol in 50 mM potassium phosphate buffer (pH 7), monitored at 340 nm. The decrease in absorbance is measured after the addition of NADH to 0.27 mM as final concentration.

Succinate dehydrogenase
The enzyme was assayed according to King (1967) [14]: 100 mM potassium phosphate buffer (pH 7.4), 0.3 mM EDTA, 0.053 mM DCIP and 100 mg of protein. The mixture was pre-incubated 10 min at 25 8C before adding 50 ml of KCN-Succinate (containing 3.25 mg/ml of KCN in 0.5 M succinate). The measure of activity was done at 625 nm.

Thiobarbutiric acid reactive substances
Lipid peroxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARS) and quantified in terms of malondialdehyde (MDA) equivalents according to the method described by Samokyszyn and Marnett (1990) [15]: 1 ml of samples was added to 1 ml solution (0.375% thiobarbituric acid and 15% trichloracetic acid in 0.25 M hydrochloric acid). The tubes were heated at 100°C during 15 min and they were cooled in the ice to stop the reaction. One then carries out a centrifugation with 1000 x g during 10 min. The reading of supernatant was made to 535 nm.

Enzyme activity expression
The specific activity of each enzyme was calculated using the following formula:
$AS = (\Delta Abs/mn \times 1000) / (\varepsilon \times [P] \times Ve)$
$\Delta Abs/mn$: Absorbance variation/minute
ε (Extinction coefficient):
$\varepsilon (H_2O_2) = 40\ M^{-1}.cm^{-1}$, for CAT
$\varepsilon (NADH) = 6220\ M^{-1}.cm^{-1}$, for SOD and GR
$\varepsilon (DCIP) = 19100\ M^{-1}.cm^{-1}$, for SDH
$\varepsilon (MDA\text{-}TBA\ complex) = 153000\ mM^{-1}.cm^{-1}$, for MDA
[P]: Protein concentration
Ve: Assay volume

Statistical Analysis
In each assay, all experimental values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed by ANOVA. Differences were considered significant at the level $p < 0.05$.

The analysis was performed with XLSTAT Version 2014.2.02

RESULTS AND DISCUSSION

Monitoring of Body Weight in Mice:
As shown in Figure 1, the body weight of all treated mice showed variations significantly different from those of the control, and control of stress. Group treated with H_2O_2 show a significant weight loss showing a low activity during treatment period shows the presence of oxidative stress. Significant increase in weight of groups (I+H) and (C+H) versus control stress, shows that NSAID Ibuprofen and L-ascorbic acid has restored the imbalance between pro-oxidant balance caused by the effect of hydrogen peroxide and antioxidant defense systems that lies at the agency level, compared the results with L-ascorbic acid and Ibuprofen in terms of weight compared to control mice and the control of stress, shows the presence of the antioxidant effect of the drug.
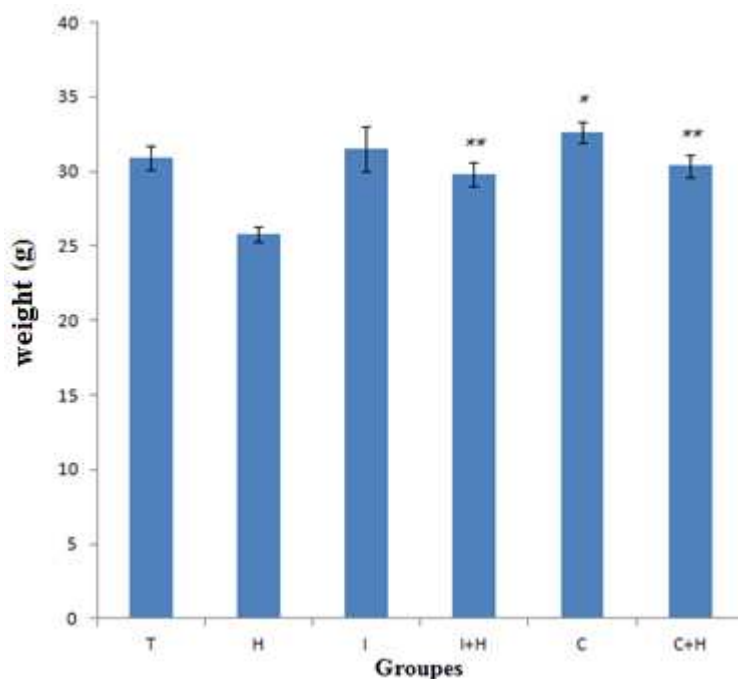
Fig. 1: weight gain in grams during 30 days of treatment
*T: Control, H: H_2O_2; I: Ibuprofen; I+H: Ibuprofen + H_2O_2; C: Vitamin C; C+H: Vitamin C + H_2O_2. *significantly different from groups (T and H) at P<0.05.**significantly different from control of stress at P<0.05. The number of mice used in each group was 6.*

The results in Figure (2; 3; 4; 5 and 6) showed that the activity of stress markers (CAT, SOD, GPx, SDH and TBARS) increased significantly in the treated group by hydrogen peroxide as compared with those of the control group. Explains presence of chronic oxidative stress in liver control stress group deduced by the presence of reactive oxygen species (ROS) generate by oxidative power of the hydrogen peroxide. Reactive oxygen species (ROS) produces oxidative stress which has been characterized in liver and includes, among several changes, an increase in the level of catalase, superoxide dismutase, glutathione peroxydase, succinate dehydrogenase and thiobarbituriquc acid reactive substances (TBARS), indicative of lipid peroxidation [25]. The level of these enzyme activities in cells is crucial for determining the steady state levels of superoxide radicals and hydrogen peroxide, superoxide dismutase converts superoxide radicals to hydrogen peroxide, which is then decomposed by catalase, and succinate dehydrogenase catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol. This occurs in the inner mitochondrial membrane by coupling the two reactions together. By one-electron reduction of oxygen give rise to reactive oxygen species (ROS) which include free as superoxide or hydroxyl radical ($OH^{\bullet}$) anion radicals. Other non-oxygen radical species can be produced, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($O_2^{\bullet-}$). The result of an imbalance between the balance of pro-oxidants and antioxidant defense systems [26].

As shown Fig (2; 3; 4; 5 and 6) activity of stress markers (CAT, SOD, GPx, SDH and TBARS) decreases significantly in the treated group by (I+H) and (C+H) as compared to the group treated with hydrogen peroxide. Explains the restoration of balance redox reactions compared to antioxidant defense in the liver, by reducing significantly the production of reactive oxygen species (ROS). also show significant protective effect against reactive oxygen species (ROS) and against their oxidative effect on the molecular level, the significant decrease in the activity of stress marker in treated groups by I and C compared to the control groups showed a protective effect against reactive oxygen species (ROS) by restoring the balance of redox reactions, this protection potential is mainly attributed to the antioxidant capacity in ascorbic acid to scavenge reactive oxygen species (ROS), these results also show that Ibuprofen also has scavenging activity ROS, which explains the antioxidant effect of NSAID Ibuprofen hide behind the other to already known. However, interpretation of these results becomes easy because administration of onely of NSAID namely, Ibuprofen in the absence of hydrogen peroxide decreases the amount of ROS in liver compared values of those recorded with hydrogen peroxide alone. The pro-oxidant effects of hydrogen peroxide and the observed protective effect of aspirin were explored by decreasing the activity of stress marker in treated groups (I+H) and (C + H) relative to control stress and from I and C compared to the control.
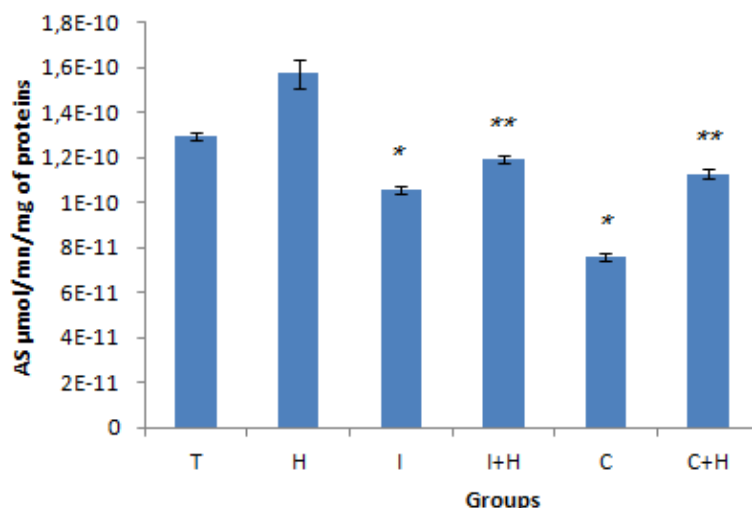

Fig. 2: Evaluated Antioxidant effect of NSAID Ibuprofen by CAT activity. T: Control, H: H_2O_2; I: Ibuprofen; I+H: Ibuprofen+ H_2O_2; C: Vitamin C; C+H: Vitamin C + H_2O_2. *significantly different from control at $P<0.05$; **significantly different from control of stress at $P<0.05$; the number of mice used in each group was 6
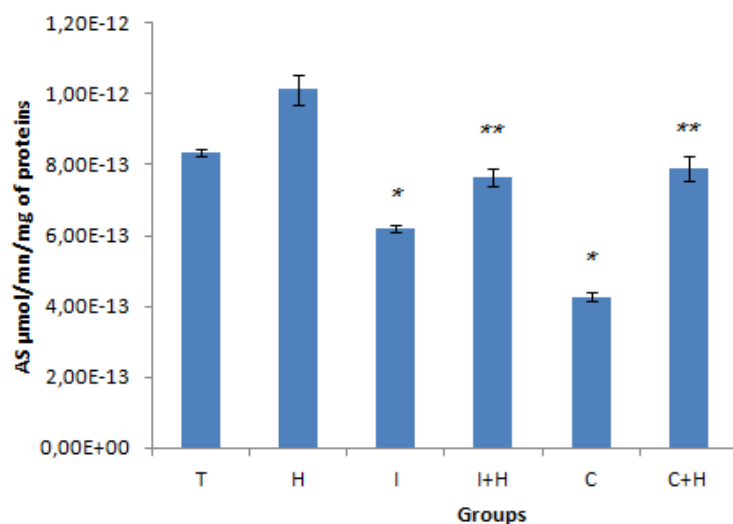

Fig. 3: Evaluated Antioxidant effect of NSAID Ibuprofen evaluated by GR activity. T: Control, H: H_2O_2; I: Ibuprofen; I+H: Ibuprofen + H_2O_2; C: Vitamin C; C+H: Vitamin C + H_2O_2. *significantly different from control at $P<0.05$; **significantly different from control of stress at $P<0.05$; the number of mice used in each group was 6
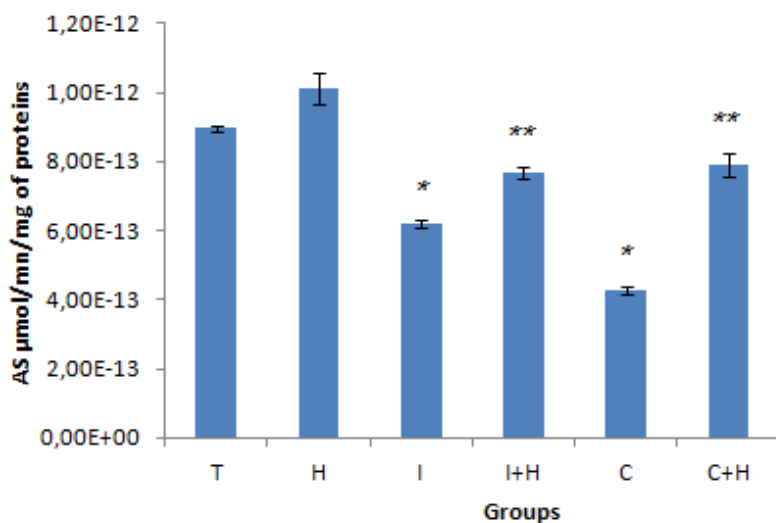

Fig. 4: Evaluated Antioxidant effect of NSAID Ibuprofen evaluated by SOD activity. T: Control, H: H_2O_2; I: Ibuprofen; I+H: Ibuprofen + H_2O_2; C: Vitamin C; C+H: Vitamin C + H_2O_2. *significantly different from control at $P<0.05$; **significantly different from control of stress at $P<0.05$; the number of mice used in each group was 6
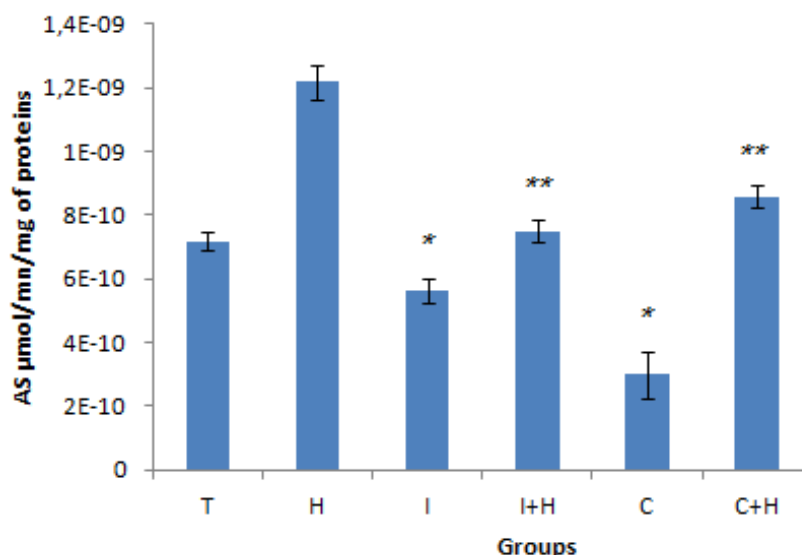
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Fig. 5: Evaluated Antioxidant effect of NSAID Ibuprofen evaluated by SDH activity. T: Control, H: H_2O_2; I: Ibuprofen; I+H: Ibuprofen + H_2O_2; C: Vitamin C; C+H: Vitamin C + H_2O_2. *significantly different from control at $P<0.05$; **significantly different from control of stress at $P<0.05$; the number of mice used in each group was 6

Fig. 6: Evaluated Antioxidant effect of NSAID Ibuprofen evaluated by TBARS activity. T: Control, H: H_2O_2; I: Ibuprofen; I+H: Ibuprofen + H_2O_2; C: Vitamin C; C+H: Vitamin C + H_2O_2. *significantly different from control at $P<0.05$; **significantly different from control of stress at $P<0.05$; the number of mice used in each group was 6
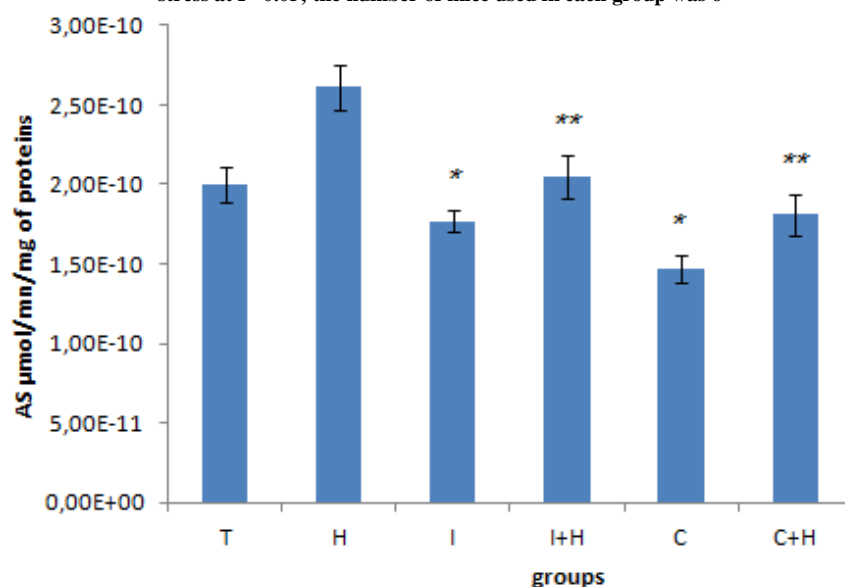
CONCLUSION

This study has shown that Ibuprofen have antioxidant effect according to the results obtained from the treatment of mice with NSAID Ibuprofen by a dose (30mg/kg) and treatment by a dose (30mg/kg) in compared with stress control group treated by H_2O_2 by a dose (100mg/kg) has demonstrated the important protective antioxidant effect of NSAID Ibuprofen against ROS compared to ascorbic acid effect. Have used vitamin C Also known as ascorbic acid name, is definitely the star of vitamins to the general public. It lends many properties, some of which are not necessarily proven. The food industry also exploits the effects recognized antioxidants ascorbic acid form of derivatives used as preservatives (E300, E301 and E302). Vitamin C is involved in many body functions: It has antioxidant properties that help to help the body fight against the accumulation of heavy metals such as lead, mercury and cadmium. Moreover, the antioxidant activity of the ascorbic acid can neutralize free radicals, thereby protecting the cells of the organism aging and for strengthening the immune defenses [27, 28, 29]

In addition, Ibuprofen has been demonstrated to inhibit chemically induced oxidative stress in animal. The protective effect of ibuprofen against ros has been presumably attributed to its ability to inhibit inflammation first and oxidation second. The anti-inflammatory and antioxidant action of Ibuprofen is believed to result from its non

specific inhibition of cyclooxygenase-2. Moreover, Ibuprofen has been reported to inhibit prostaglandin biosynthesis by inhibiting cyclooxygenases (COXs) have two major subtypes COX-1 and COX-2 [5], COX -2 is inducible and mediates inflammation response [30]. in the state of inflammation there to increase the oxidation reaction in the body due to the activation of metabolism which explains the presence ros in the state of inflammation. in addition, reactive oxygen species can also be produced by inflammatory cells as well as many other cellular sources [31,32]. Metabolism of a variety of stressful chemical molecule also leads to generation of ros [32,33]. The main mode of action is irreversible inhibition of cyclooxygenase activity by acetylating serine in the active site of the enzyme. This causes the inhibition of prostaglandin and thromboxane synthesis. Cox-2 is rapidly inducible by e.g. inflammatory stimuli and contributes to inflammatory responses, which explains the antioxidant effect of drug inhibition hide behind cox-2. We demonstrated a dose of Ibuprofen that inhibited oxidative power of hydrogen peroxide and production of reactive oxygen species is a (30mg/kg). In conclusion, the results show clearly on the protective antioxidant effect of Ibuprofen against the intoxicate H_2O_2 and ROS generation, in order to strengthen the system of antioxidant defense and inhibited the activation pathways of the molecules responsible for causing oxidative stress.

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