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Evaluation of Diclofenac effect on oxidative stressed mice

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ABSTRACT

Diclofenac (2-[(2,6-dichlorophenyl)amino]benzene acetic acid) Non-steroidal anti-inflammatory drug (NSAID) is among the most frequently used medical drug as analgesic, anti-inflammatory and antipyretic effect. This study aims to evaluate if Diclofenac has an antioxidant effect, relative to its analgesic antipyretic and anti-inflammatory activities, or it possesses a cytotoxic potential. Oxidative stress was induced by intraperetoneal injection of peroxide hydrogen (H_2O_2), and then a comparative study is made concerning the activities of the antioxidant enzymes SOD, CAT, GR, SDH as well as MDA lipid peroxidation levels in liver. An decrease in SOD, CAT, GR, SDH activity and MDA lipid peroxidation in mice treated with H_2O_2 accompanied by Diclofenac; compared to the group treated by L-ascorbic acid + H_2O_2 showed that diclofenac show good antioxidant effect. Moreover this study has suggested that acetylsalicylic acid induced anti-inflammatory effects in liver mediated by decrease oxidative stress and restore redox reaction of metabolism.

***Key words**: Diclofenac, Oxidative stress, Antioxidant.*

INTRODUCTION

Diclofenac, (2-[(2,6-dichlorophenyl) amino] benzeneacetic acid) is a non-steroidal anti-inflammatory drug with good analgesic properties, that are used for the treatment of mild to moderate pain, fever, and inflammation caused by arthritis or ankylosing spondylitis. NSAIDs work by reducing the production of prostaglandins, chemicals that cause pain, fever and inflammation[1]. They are also used to treat a migraine headache attack. Their therapeutic effects result mostly from the inhibition of cyclooxygenase (COX), an enzyme involved in the production of prostaglandins which have a strong propensity for inducing inflammation[2,3]. It is well known that COX exists in two isoforms, COX-1 and COX-2, which are regulated differently[4]. COX-1 provides cytoprotection in the gastrointestinal (GI) tract whereas inducible COX-2 mediates inflammation[5].

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COX-2 is an attractive target for medicinal chemists as it is expressed only in few normal tissues and is greatly up regulated in inflamed tissues as well as many premalignant and malignant tumors. Since, most of the NSAID in the market show greater selectivity for COX-1 than COX-2[6] the Diclofenac moiety has been extensively exploited by the organic and medicinal chemist to synthesize potential therapeutic agents such as antibacterial, antitumor and antituberculosis[7,8]. Several studies have shown that nonselective COX-2 inhibitors had beneficial effects on atherosclerosis progression inhibiting leukocyte aggregation, decreasing inflammatory cytokines, inhibiting superoxide radicals from macrophages and lowering plasma cholesterol levels[9,10].

The objective of the present research was to examine and test precisely antioxidant activity of NSAID Diclofenac and even the protective effect at long-term of treatment in liver of mice, compared and analyzed theactivityofincreasingstress markers that suits hydrogen peroxide by oxidation, and reduction of the activity of stress markers suitable for treatment by Vitamin C, with the decrease of the activity markers stress in the group streated by Diclofenac.

MATERIEL AND METHODS

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	Diclofenac	30 mg/kg
4	6	Diclofenac + 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, USA).

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].

Glutathione reductase

The assay of Di ilio*et al.*,[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 Paolett*i et al.,*[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[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 bySamokyszyn and Marnett[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:

$AS = (\Delta Abs/mn \times 1000) / (\varepsilon \times [P] \times Ve)$

ΔAbs/mn: Absorbance variation/minute

ε (Extinction coefficient):

ε (H_2O_2) = 40 $M^{-1}.cm^{-1}$, for CAT

ε (NADH) = 6220 $M^{-1}.cm^{-1}$, for SOD and GR

ε (DCIP) = 19100 $M^{-1}.cm^{-1}$, for SDH

ε (MDA-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:

According to 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 (Diclofenac +H_2O_2) and (Vitamin C+H_2O_2) versus control stress, shows that NSAID Diclofenac 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 Diclofenacin terms of weight compared to control mice and the control of stress, shows the presence of the antioxidant effect of the drug.

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 thiobarbituric acid reactive substances (TBARS), indicative of lipid peroxidation[16]. 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 oxygengiverise toreactive oxygen species (ROS) which include free as superoxide or hydroxyl radical (OH°) anion radicals. Other non-oxygen radical species can be produced, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($O2^{-}$). The result of an imbalance between the balance of pro-oxidants and antioxidant defense systems[17].

As shown in Fig (2; 3; 4; 5 and 6) activity of stress markers (CAT, SOD, GPx, SDH and TBARS) decreases significantly in the treated group by (Diclofenac $+H_2O_2$) and (Vitamin C$+H_2O_2$) 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 D 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 acetyl salicylic acid also has scavenging activity ROS, which explains the antioxidant effect of NSAID Diclofenachide behind the other to already known. However, interpretation of these results becomes easy because administration of only of NSAID namely, Diclofenac 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 Diclofenac were explored by decreasing the activity of stress marker in treated groups (Diclofenac $+H_2O_2$) and (Vitamin C$+H_2O_2$) relative to control stress and from Diclofenac and Vitamin C compared to the control.

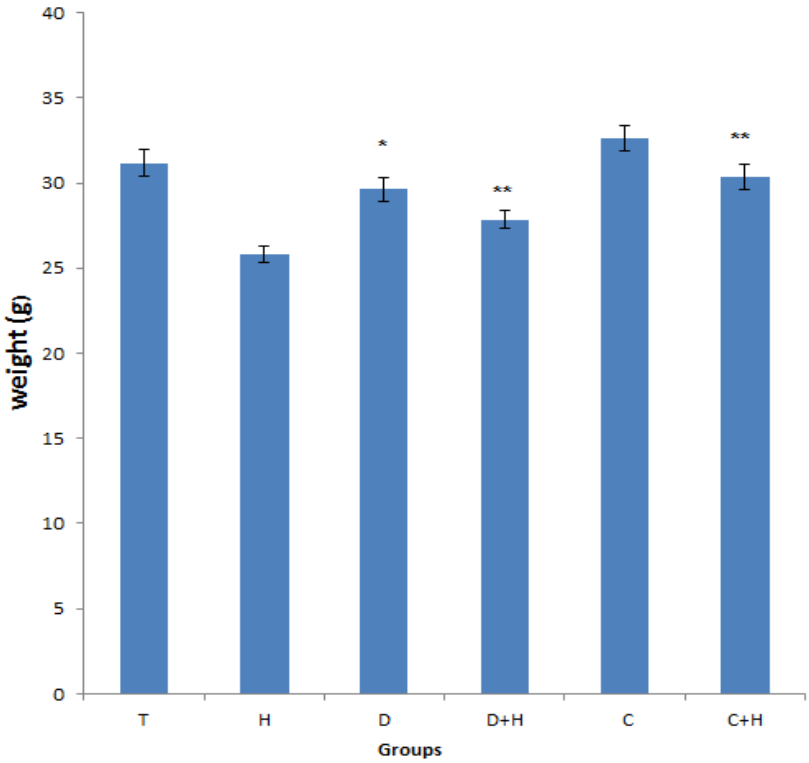

Fig. 1: weight gain in grams during 30 days of treatment. T: Control, H: H2O2; D: Diclofenac; D+H: Diclofenac+ H2O2; C: Vitamin C; C+H: Vitamin C + H2O2. *significantly different from groups (T; H) at P<0.05.**significantly different from control of stress at P<0.05. The number of mice used in each group was 6.

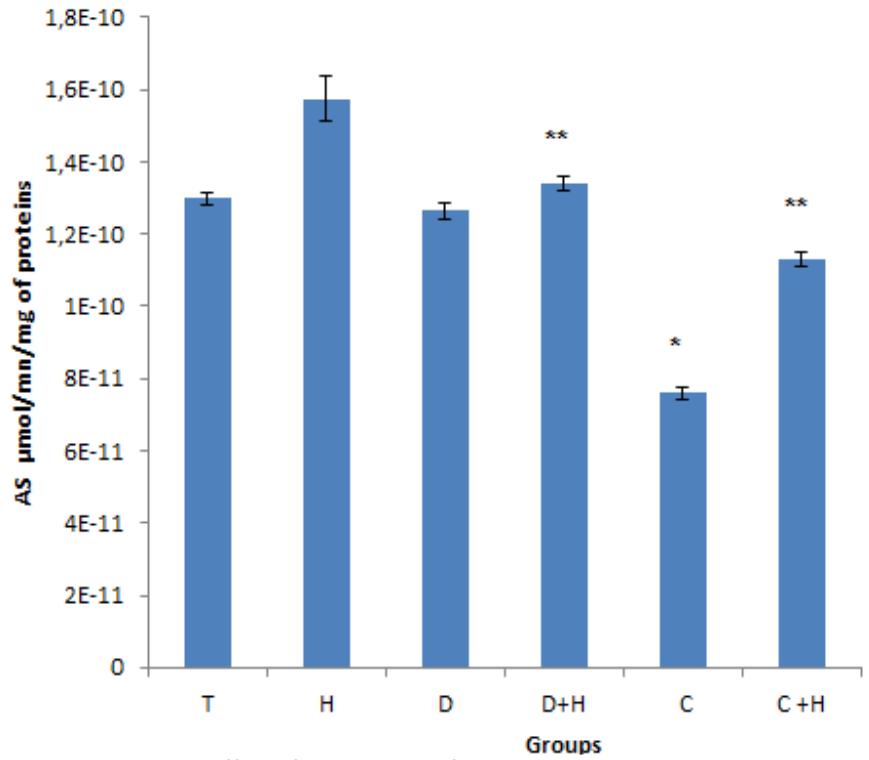

Fig. 2: Evaluated Antioxidant effect of NSAID Diclofenac by CAT activity. T: Control, H: H2O2; D: Diclofenac; D+H: Diclofenac+ H2O2; C: Vitamin C; C+H: Vitamin C + H2O2. *significantly different from control at P<0.05; **significantly different from control of stress at P<0.05; the number of mice used in each group was 6.

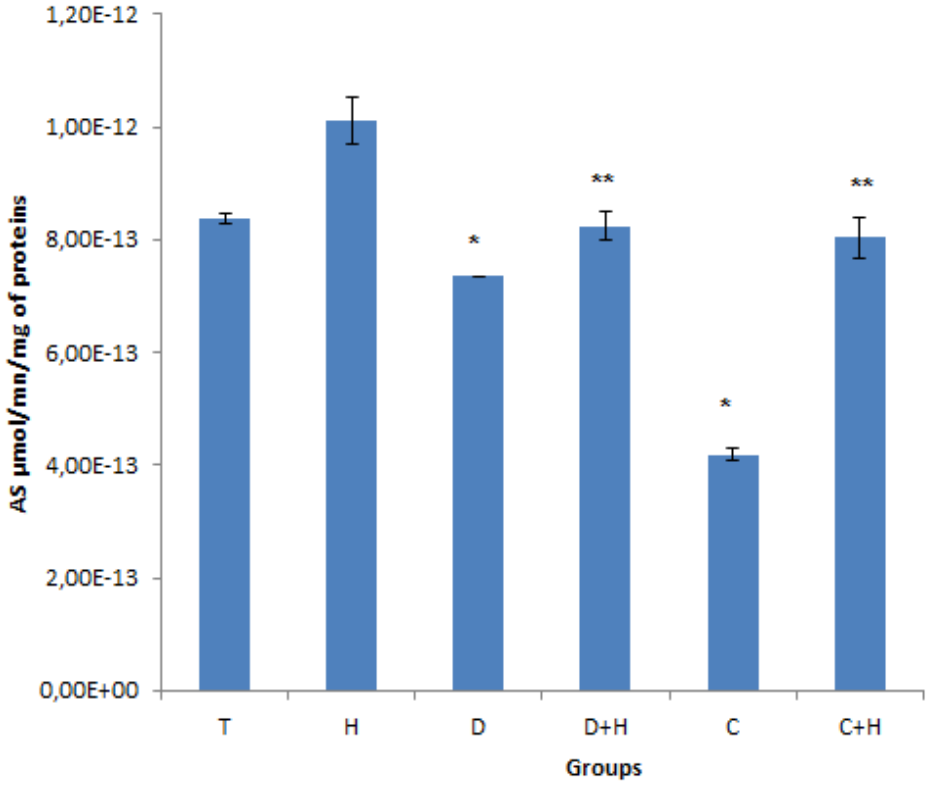

Fig. 3: Evaluated Antioxidant effect of NSAID Diclofenac evaluated by GR activity. T: Control, H: H2O2; D: Diclofenac; D+H: Diclofenac+ H2O2; C: Vitamin C; C+H: Vitamin C + H2O2. *significantly different from control at P<0.05; **significantly different from control of stress at P<0.05; the number of mice used in each group was 6.

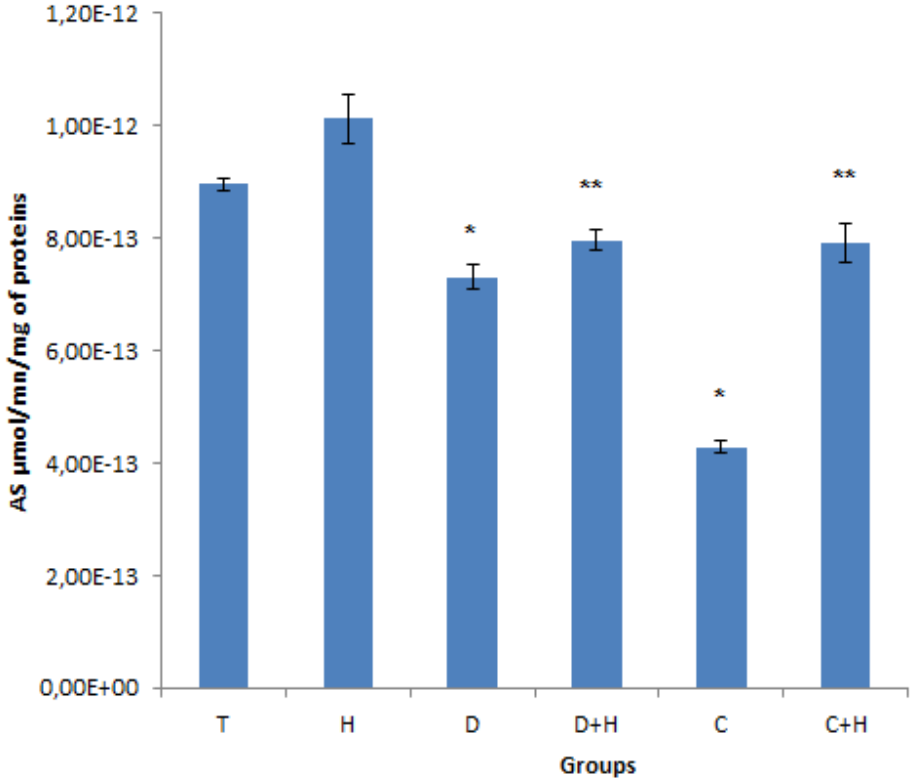

Fig. 4: Evaluated Antioxidant effect of NSAID Diclofenac evaluated by SOD activity. T: Control, H: H2O2; D: Diclofenac; D+H: Diclofenac+ H2O2; C: Vitamin C; C+H: Vitamin C + H2O2. *significantly different from control at P<0.05; **significantly different from control of stress at P<0.05; the number of mice used in each group was 6.

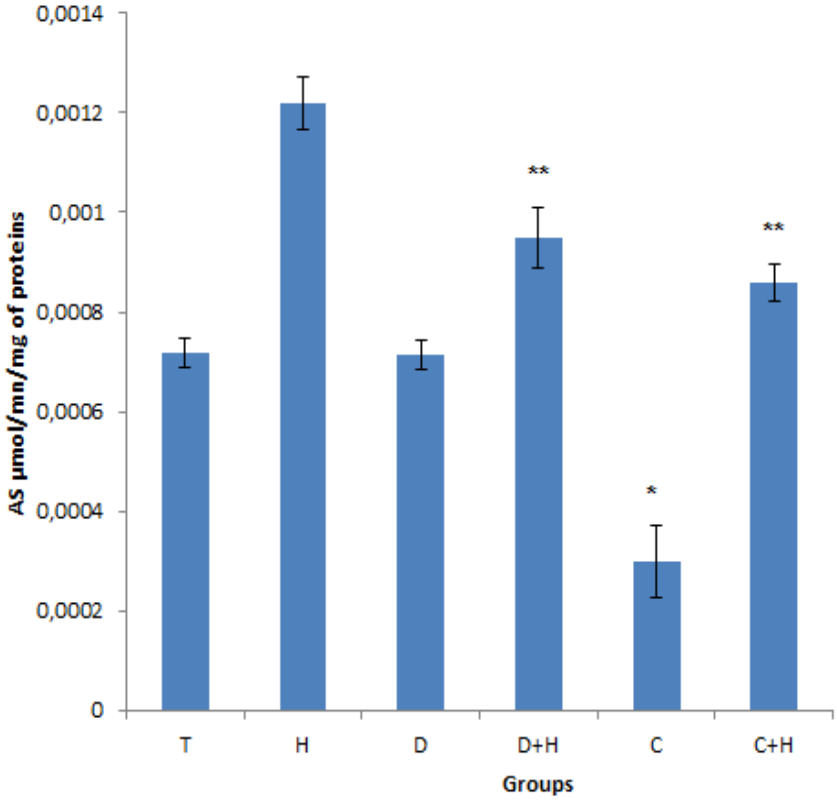

Fig. 5: Evaluated Antioxidant effect of NSAID Diclofenac evaluated by SDH activity. T: Control, H: H2O2; D: Diclofenac; D+H: Diclofenac+ H2O2; C: Vitamin C; C+H: Vitamin C + H2O2. *significantly different from control at P<0.05; **significantly different from control of stress at P<0.05; the number of mice used in each group was 6.

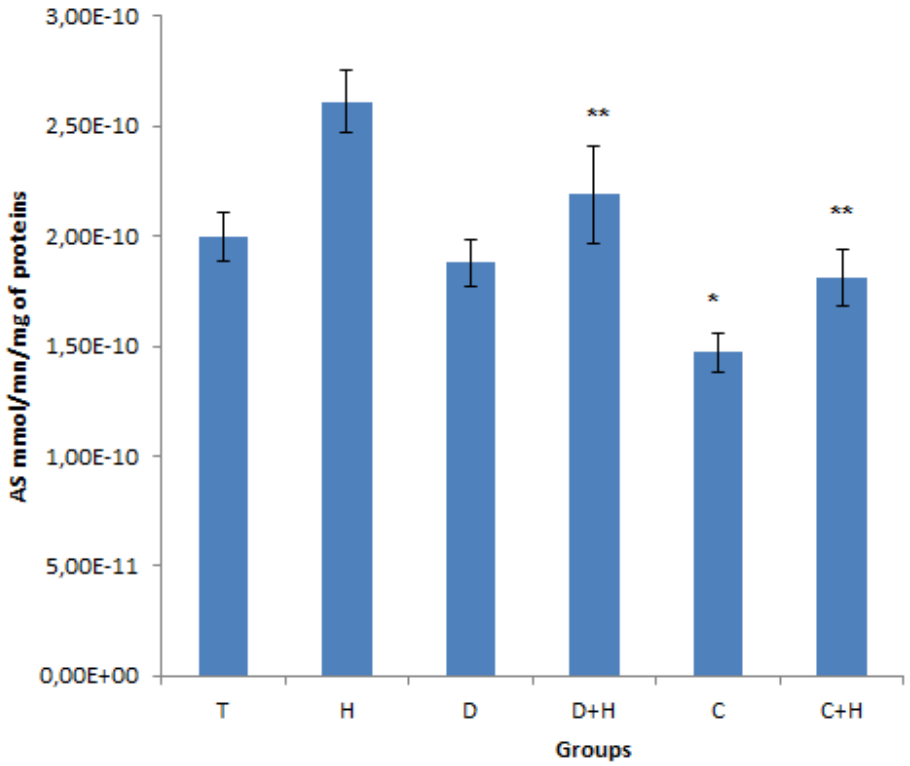

Fig. 6: Evaluated Antioxidant effect of NSAID Diclofenac evaluated by MDA activity. T: Control, H: H2O2; D: Diclofenac; D+H: Diclofenac+ H2O2; C: Vitamin C; C+H: Vitamin C + H2O2. *significantly different from control at P<0.05; **significantly different from control of stress at P<0.05; the number of mice used in each group was 6.

CONCLUSION

The present study demonstrates the protection against hydrogen peroxide-mediated liver toxicity by NSAID Diclofenac, an inhibitor of cyclooxygenase. It also showed that that NSAIDDiclofenac is a potent antioxidative agent which markedly reduced oxidative stress damages by establishing the redox homeostasis. This might be useful for the prevention of oxidative stress.

One way to confirm these results at the molecular level, a necessary task, is to examine the effect of these treatments on the gene transcription of the different enzyme as biomarkers of oxidative stress. These kinds of studies are in progress in our laboratory.

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REFERENCES

1. Smalley, W.E., Ray, W.A., Daughterty, J.R., Griffin, M.R., Nonsteroidal anti-inflammatory drugs and the incidence of hospitalization for peptic ulcer disease in elderly persons, *Am. J. Epidemiol*. **141:** 539–545 (1995).
2. Tomisato, W., Tanaka, K., Katsu, T., Kakuta, H., Sasaki, K., Tsutsumi, S., Hoshino, T., Aburaya, M., Li, D., Tsuchiya, T., Suzuki, K., Yokomizo, K. and Mizushima, T., Membrane permeabilizationbynon-steroidal anti-inflammatory drugs, *Biochem. Biophys. Res. Commun.***323:** 1032–1039 (2004).
3. Burke, A., Smyth, E., FitzGerald, G.A., Analgesic–antipyrectic agents: pharmacotherapyof gout, in: L.L. Brunton (Ed.), Goodman & Gilman's The Pharmacological Basisof Therapeutics, 11th ed., McGraw-Hill, USA, ch. 26 (2006).
4. Dannhardt, G., Kiefer, W., *Eur. J. Med. Chem.***36:** 109 (2001).
5. Almansa, C., Alfon, J., Arriba, A. D., Cavalcanti, F., Escamilla, I., Gomez, L.,

Miralles, A., Soliva, R., Bartolli, J., Carceller, E., Merlos, M. and Garcia-Rafanell, J. J., *Med. Chem.***46:** 3463 (2003).

6. Jackson, L. M. and Hawkey, C. J., *Expert Opin. Invest. Drugs***8:** 963 (1999).
7. Dastidar, S.G., Kristiansen, J., Molnar, J., Martins, M. and Amaral, L., *Int. J. Antimicrob. Agents***14:** 249 (2000).
8. Sriram, D., Yogeeswari, P. and Devakaram, R., Bioorg. *Med. Chem.***14:** 3113 (2006).
9. Konstandinidou, M., Kourounakis, A. and Yiangou, M., Anti-inflammatoryproperties of diclofenac transition metalloelement complexes. *Journal Inorganic Biochemistry***70 (1):** 63–69 (1998).
10. Nawawi, H., Osman, N.S., Yusoff, K. and Khalid, A., Reduction in serumlevels of adhesion molecules, interleukin-6 and c-reactive protein followingatorvastatin treatment. *Hormone Metabolism Research***35(2):** 479–485 (2003).
11. Zentella de Pina, M., Sandoval-Montiel, A., Serrano-Alessandri, L., Montalvo-Jave, E., Zentella-Dehesa, A. and Pina, E, "Ethanol-mediated Oxidative Changes in Blood Lipids and Proteins Are Reversed by Aspirin-like Drugs", *Archi Med Res,* **38:** 269-275 (2006).
12. Bradford, M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding", *Anal Biochem,* **72:** 248-254 (1976).
13. Aebi, H., Catalase *in vivo*. *Meth. Enzymol.***105:** 121-126 (1984).
14. Di Ilio, C., Polidoro, G., Arduini, A., Muccini, A., Federici, G., Glutathione peroxidase,Glutathionereductase, gluthatione S-transferase and gamma_glutamyltranspeptidase activities in the human early pregnancy placenta. *Biochem. Med.*,**29:** 143-148 (1983).
15. Paoletti, F., Aldinucci, D., Mocali, A., Carparrini, A., A sensitive spectrophotometric method for the determination of superoxide dismutase in tissue extracts. *Anal. Biochem.*,**154:** 526–541 (1986) .
16. Samokyszyn, V.M. and Marnett, L.J., Inhibition of liver microsomal lipid peroxidation by 13-cisretinoic acid Free, *RadicBiol Med.*, **8:** 491-6 (1990).
17. King, T.E., Preparation of succinate cytochroine c reductase and cytochroine b-cl particles and reconstitution of succinate-cytochrorne c reductase, in: R. Estabrook (ed.) Meth. Enzymol., 225. Academic Press. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokio.**10:** 216-231(1967).