ejpmr, 2018,5(8), 130-136

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTI-INFLAMMATORY PROPERTY OF NOVEL ISOINDOLE LEAD COMPOUNDS AND ESTIMATION OF LD50 OF THE MOST ACTIVE ENTITY

Madani A. M.[1], Aimun A. E. Ahmed[2]*, Tilal Elsaman[3], EI-Hadiyah T. M.[4] and Hatem A. Abdel-Aziz[5]

[1]Pharmacologist and Specialist of Clinical Pharmacy, Cardiac Center, Omdurman Teaching Hospital, Sudan.
[2]Pharmacology Department, Faculty of Pharmacy, Omdurman Islamic University, Khartoum Sudan, P.O. Box 2587. and Pharmacology Department, Faculty of Medicine, Albaha University, Albaha Saudi Arabia, P.O. Box 1988.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Omdurman Islamic University, Khartoum Sudan, P.O. Box 2587.
[4]Department of Pharmacology, Faculty of Pharmacy, International University of Africa, Sudan, P.O. Box 83, Sudan.
[5]Department of Applied Organic Chemistry, National Research Center, Dokki, Giza 12622, Egypt.

***Corresponding Author: Dr. Aimun A. E. Ahmed**

Pharmacology Department, Faculty of Pharmacy, Omdurman Islamic University, Khartoum Sudan, P.O. Box 2587 and Pharmacology Department, Faculty of Medicine, Albaha University, Albaha Saudi Arabia, P.O. Box 1988.

Article Received on 09/06/2018 **Article Revised on 29/06/2018** **Article Accepted on 19/07/2018**

ABSTRACT

Background: Isoindole derivatives serve as an important source for lead compounds with numerous pharmacological uses. Objectives: The study aimed to investigate the anti-inflammatory property for newly synthesized six isoindole derivatives; TIND-1, TIND-7, TIND-10, TIND-11, TIND14 and TIND-15, and to estimate LD50 of most promising one. **Methods:** The carrageenan-induced paw edema model in rats used to investigate the anti-inflammatory activity of the tested compounds and to compare between them and the standard drug using inhibition of edema as a pharmacological parameter. Whilst the LD_{50} was estimated for TIND-7, using OEDC-AOT 425 guideline for acute oral toxicity on rats, in vivo. **Results:** All tested compounds exhibited various time-dependent anti-inflammatory activity, through different period of time; first hour, significant effect was revealed for TIND-14 ($P<0.01$) and TIND-15 ($P<0.001$), whilst at third hour, TIND-7 exhibited 71%, TIND-14 and TIND-15 demonstrated highest activity. Moreover, at fifth hour TIND-7 produced 100% inhibition. The estimated LD50 for the most active compound (TIND-7) was more than 2000 mg/kg. **Conclusion:** All tested compounds showed variety of anti-inflammatory activity against carrgenan-induced paw edema on rats, in vivo, however TIND-7 was the most promising compound ever, with reasonable margin of safety estimated as LD50.

KEYWORDS: Anti-inflammatory, Isoindole, Paw-edema, Diclofenac, LD50, Protocol 425.

INTRODUCTION

Inflammation is protective set of interactions arise in response to trauma, infection, post ischemia, toxins and auto-immune injury, and involves immune cell blood vessels (Dhar et. al., 2014; Lam et al., 2015) and molecular mediators (Farhangi et. al., 2013; Dubois et. al., 2014). However, uncontrolled inflammation can cause suffering such as organ damage, disabilities and pain that need use of anti-inflammatory medications for treatment of undesired signs of inflammation. Available anti-inflammatory drugs such as non-steroidal ant-inflammatory drugs (NSAIDs) have potential side effects such as gastrointestinal bleeding (Libby et. al., 2007), renal and cardiovascular complications (Harirforoosh et. al., 2013) and worsening of asthma symptoms. Recently isoindole derivatives became a target for promising compounds with anti-inflammatory and immunomodulatory effects. Isoindoles are particularly useful for treating diseases caused or aggravated by excessive or unregulated levels of tumor necrosis factor alpha (TNF-α), interleukin-beta (IL-β), interleukin-10 (IL-10) or T-lymphocytes (T-cells). Isoindole is basically used as anti-inflammatory (Klaus et. al., 2013), anticancer, antiviral and antibacterial (Kumar et. al., 2013). Famous isoindole drug thalidomide, developed in late 1950s used for treatment of morning sickness in pregnancy withdrawn later due to risk of teratogenicity. Great efforts were done during last years to discover new thalidomide-analogues for cancer treatment (Armoiry et. al., 2008) or other isoindole entities with desirable anti-inflammatory activity and reasonable toxicity (Ferenc Csende et. al., 2013). On the other hand, recently series of synthetic isoindole derivatives were synthesized and expected to have promising anti-inflammatory activity (Abdel-Aziz et. al., 2014). This study was set out to investigate the anti-inflammatory property of these compounds and to estimate LD50 of most promising one.

2. MATERIALS AND METHODS

2.1. Animals housing

Swiss albino rats of either sex purchased from animals houses of National Research Centre, Khartoum, Sudan were housed in standard cages and room temperature was approximated to $24C^{O}$ ($\pm 1C^{O}$). Animal fed with standard diet and supplied with continuous source of purified water. Cages cleared from waste and rats' general health was daily observed.

2.2. Ethical considerations

All experiments done after obtaining ethical clearance permission from institutional animal committee, pharmacology department, faculty of pharmacy, OIU, under the serial no (OIU/I.A.E.C./ Exp. Ph., TOX. /2015/0.01).

2.3. Anti-inflammatory activity investigations

The Carrageanan-induced paw edema model was used to assess the anti-inflammatory activity of the Tested TIND compounds and standard diclofenac sodium, where 32 rats (120-150 g) were randomly divided into 8 groups of four rats each. After being fasted over night the tested compounds were dissolved in distilled water and administered orally via gastric gavage to rats. Group I received 1ml/100mg/kg of distilled water and served as (-ve control), group II received 25mg/kg diclofenac sodium and served as (+ve control), the tested groups; III, IV, V, VI, VII and VIII received 25mg/kg of TIND-1, TIND-7, TIND-10, TIND-11, TIND-14 and TIND-15 respectively. One hour later paw edema was induced by injection of 0.1ml of (1%) freshly prepared carrageenan suspension. Thickness of Paw edema was measured and registered using Vernier-caliper for each rat at first, third and fifth hour intervals. Percentage of inhibition of carrageenan induced-paw edema was calculated according to the following equation:

$$\% \text{ of inhibition} = 100\times [1- (x_2-x_1)/(y_2-y_1)]$$

Where x_1 is the thickness of rats paw before administration of carrageenan in tested or standard compounds groups, x_2 is the thickness of rats paw after administration of carrageenan in the test or standard compounds groups, y_1 is the thickness of rats paw before the administration of carrageenan in the control group and y_2 is the thickness of rats paw after administration of carrageenan in the control group.

2.4. Median lethal dose (LD50) estimation

Acute oral toxicity was performed on rats according to guidelines for testing of Chemicals prepared by the Organization for Economic Co-operation and Development (OECD) (OECD., 2001) guided by the computerized acute oral toxicity (AOT-425) program to facilitate direct calculation of LD50 of TIND-7 compound.

2.4.1. Limit test

The software-guided Limit test started with one rat that received 2000 mg/kg of TIND-7 compound orally via gastric gavage. The rat observed for pre-designed time interval (short- term, 48 hours and long – term 14 days) and results recorded as mentioned within the guidelines as (O=dead, X= Alive) then the software instructions were followed till the stopping criteria appeared on the screen indicating the end point of the limit test, so we stopped dosing and start conducting the main test.

2.4.1. Main test

Five rats were used, according to doses suggested byAOT-425program, the 1st rat received 175mg/kg, the 2nd rat given 550 mg/kg and the 3rd, 4th and 5th rats given 2000 mg/kg orally via gastric gavage. All rats observed after dosing with special attention during the first 30 minutes, 4 hours and then daily for 14 days. All observations recorded in separate sheet for each rat daily. Observations included; mortality rate and changes in skin, fur, eyes and mucous membranes besides, behavioral patterns, such as tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and respiration rate.

2.5. Statistical analysis

Statistical calculations were done using Prism 5.0 for windows computer program, version 5.01 (USA). Data were represented as mean ± standard error of mean (SEM). Unless, indicated all results were analyzed using One-way Analysis of Variance (One way-ANOVA) followed by Dunnett's test to calculate statistical differences among groups and the difference considered significant at $P\leq 0.05$. Moreover, the special protocol relevant software (AOT-425stat) was used to estimate the LD50 for the most active compound.

3. RESULTS

3.1. Anti-inflammatory activity results

During first hour time interval both compounds; TIND-1 and TIND-7 showed similar activities to the standard diclofenac, in addition to, higher activities were reported for TIND-10, TIND-14 and TIND-15; whilst TIND-11 showed no activity (Fig.1A).

During third hours compounds; TIND-7, TIND-14 and TIND-15 were the most highly active (Fig.1B).

During fifth hours, TIND-7 showed highest activity comparable to the standard and TIND-1 had typical activity to that of diclofenac as shown in (Fig.1C).

3.2. Estimation of LD50 and rats' observations

According to calculations obtained from Limit and Main test of Acute Oral Toxicity (Guideline 425) experiments guided by Statistical Program (Version:1.0, 2001), the LD50 was estimated to be more than 2000 mg/kg (Fig. 2, 3).

Short and long-term inspections revealed that, the treated rats with TIND-7 experienced increase in respiration rate (gasping of air) just during the first 30minutes at all doses. This change in respiration rate was found to be higher in rats treated with 2000 mg/kg. Whilst no other changes were observed for other wellness parameters during the study periods (Table 2).

TABLES

Table 1: The six synthetic isoindole derivatives compounds with their main features.

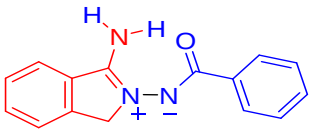
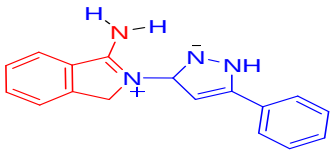
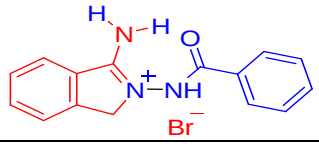
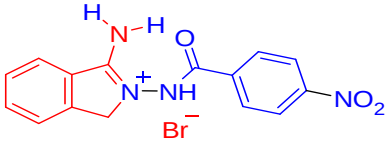
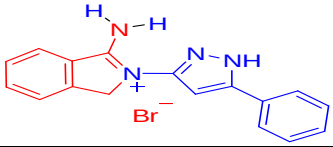
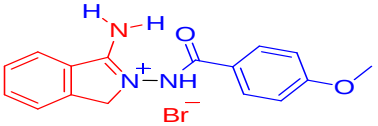
Code	Chemical Structure	Melting Point	Molecular weight
TIND-1		230-232	252
(3-amino-1H-isoindolium-2-yl)(benzoyl)amide			
TIND-7		255-257	275
5-(3-amino-1H-isoindolium-2-yl)-3-phenyl-2,5-dihydropyrazol-1-ide			
TIND-10		297-299	332
3-amino-2-benzamido-1H-isoindolium bromide			
TIND-11		300-302	378
3-amino-2-(4-nitrobenzamido)-1H-isoindolium bromide			
TIND-14		358-360	355
3-amino-2-(5-phenyl-1H-pyrazol-3-yl)-1H-isoindolium bromide			
TIND-15		325-327	362
3-amino-2-(4-methoxybenzamido)-1H-isoindolium bromide			

Table 2: Observations for acute doses of TIND-7 during different time intervals.

Observation	30 min		4 hrs		24 hrs		48 hrs		2 wks		1 wk	
	C	T	C	T	C	T	C	T	C	T	C	T
Skin & Fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes change	N	N	N	N	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N	N	N	N	N
Convulsion	N	N N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	
Diarrhea	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Coma	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Respiration	Nil	**Ab Nil**	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

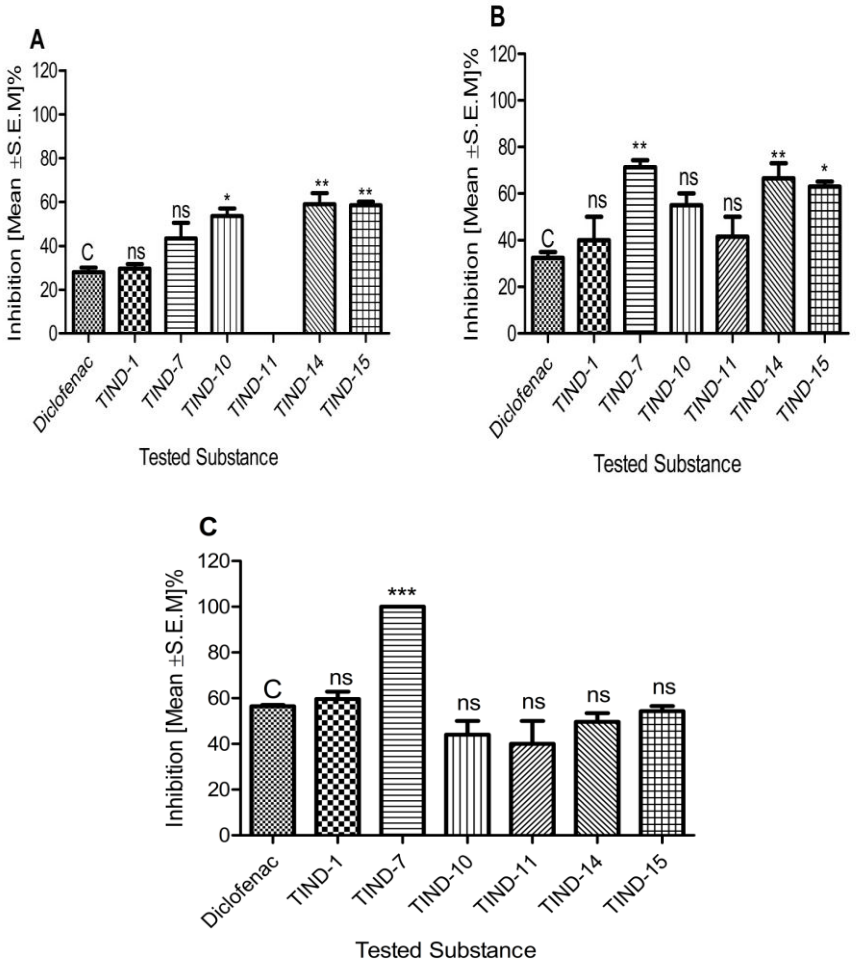


Figure 1: The comparison between the six synthetic compounds and the standard diclofenac; at three different period of time; 1hour (A), 3hours (B) and 5hours (C); against carrageenan-induced pew edema, in vivo on rats. The presented data were the % inhibition [mean ± S.E.M], whilst data were obtained from 4-6 animals, during the three different time intervals (1, 3 and 5 hours). C; control, *P≤0.05, **P≤0.01, ***P≤0.001, ns; non-significant statistically.

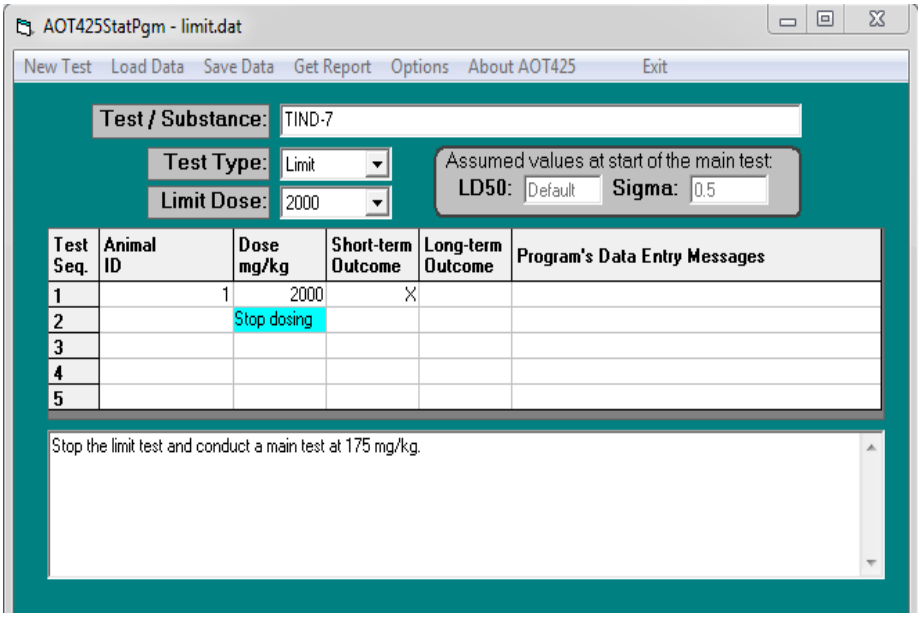


Figure 2: Screen shot of the AOT 425 program showed the limit test, short-term. Stopping criteria appears after one dose of TIND-7 at 2000 mg/kg for one rat, and death of the rat represented as letter (X).

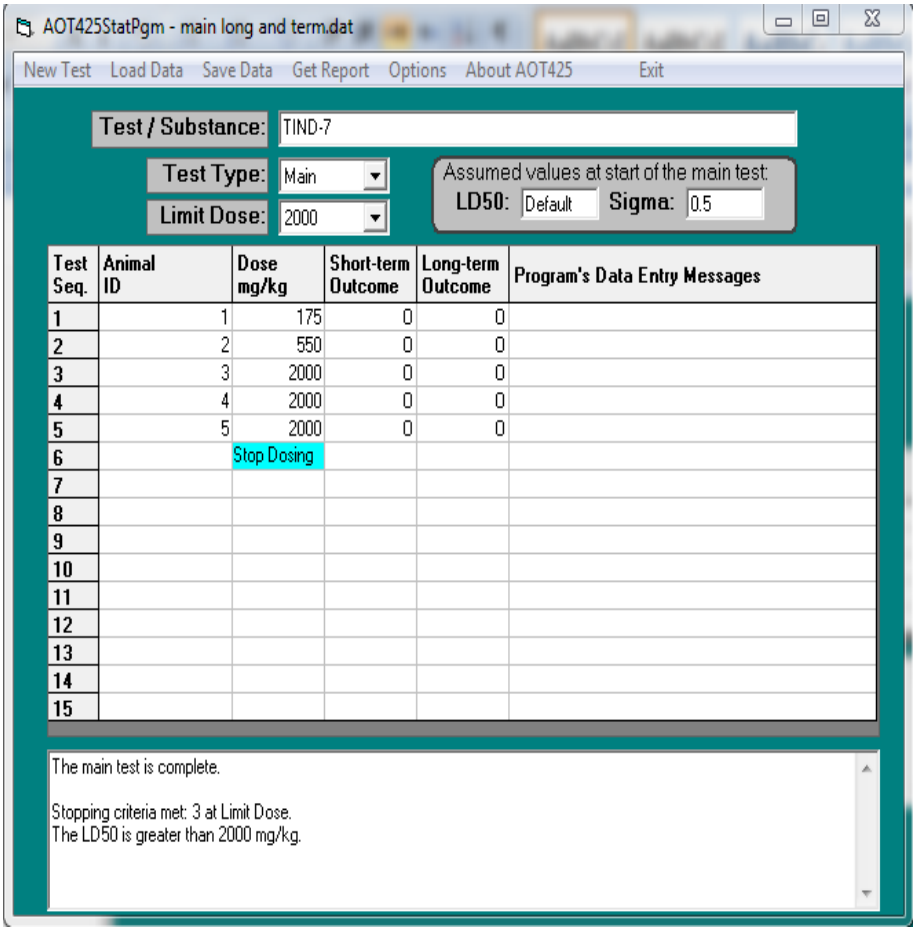


Figure 3: Screen shot of the AOT 425 program show the short and long term of the main test of TIND-7 at 175 mg/kg, 550 mg/kg and 2000 mg/kg doses. All five treated rats were alive after short term (48 hours) and long-term (14 days) inspection and represented with letter (O).

4. DISCUSSION

A set of six promising synthetic isoindole derivatives lead compounds were synthesized and tested for anti-inflammatory activity by inhibition against carregenen-induced paw edema of rats, in vivo, besides, the estimation of LD50 with principal toxicity characteristics change observations of the most promising compound was done using OECD-AOT425 protocol.

All six tested compounds, at dose of 25 mg/kg, produced various degrees of reduction of carrageenan- induced paw edema, this indicated that, our compounds have remarkable anti-inflammatory activity; and these finding were in line with Al-Qaisi and her workers (Al-Qaisi et. al., 2014) who proved that isoindole nucleus possess such activity.

The present results revealed that, TIND-1 exhibited similar inhibition percentage to standard diclofenac during first hour, this can be taken as an evidence that our compound have similar relative potency to diclofenac, this contradicts with Jordanian study used isoindole compounds where their results give variety of inhibition percentage ranged from 10 to 73% (Al-Qaisi et. al., 2014). Compounds TIND-10, TIND-14 and TIND-15 exhibited significant inhibition percentage, whilst TIND-7 produced the most significant effect. This proved that TIND-10, TIND-14 and TIND-15 have potential effect during the first stage of inflammation. In the literature carrageanan-induced paw edema is biphasic event, the initial phase occur during first hour and not inhibited by non-steroidal ant-inflammatory drugs (Posadas et. al., 2004), whilst second phase occur after second hour and terminated by the six hour (Salvemini et. al., 1996). TIND-11 exhibited no activity during the first hour, and this slow onset of action may be attributed either to variation in pharmacokinetic character or to the fact that TIND-11 delayed action of inflammation is related to production of prostaglandins in high concentration, nitric oxide and pro-inflammatory cytokines. This variation in onset of action and potency of our compounds may be of great value for treatment of inflammation diseases with variation in intensities.

Anti-inflammatory activity of our compounds increased during the third hour which exhibited the peak of anti-inflammatory activity of TIND-14 ($p<0.01$) and TIND-15 ($p<0.05$), and also exhibited the late onset of action of TIND-11 as active anti-inflammatory compound, this may be attribute to pharmacokinetic properties of these compounds or the biphasic character of carrageenan-induced paw edema model (Mansouri et. al., 2015).

Inhibition of carrageenan-induced paw edema continued to increase also for TIND-7 and this taken as evidence to elevation of anti-inflammatory potency, which reached 71.3%. These findings were higher than that found in Derle and his colleagues' study who reported 65.3% for mefenamic acid (Derle et. al., 2008) and that obtained by Kumar (Kumar et. al., 2009) and his co-workers whom reported 70.2% for ibuprofen. This indicated potentiality of TIND-7 as promising novel anti-inflammatory entity compared to conventional NSAIDS.

At fifth hour TIND-7 inhibition of carrageenan-induced paw edema elevated to reach 100% and this revealed that TIND-7 potency as anti-inflammatory ($P<0.001$) was almost twofold of that of diclofenac in our study. Percent obtained in this work demonstrated TIND-7 potency was higher than that reported by many studies such as Cong study who reported 81% for naproxen and 54% for indomethacin (Cong et. al., 2015). These findings revealed that TIND-7 could added to effort of drug discovery, searching for lead chemical entities with high potent anti-inflammatory to replace the use of conventional NSAIDs with its documented toxicity.

The estimated LD50 of the most active compound (TIND-7) by AOT 425 was greater than 2000 mg/kg. This finding demonstrated significant margin of safety comparable to the standard Diclofenac according to the Globally Hormonized System (GHS) of classification and labeling of chemicals (Nath and Yadav 2015). Whilst European Medicine Agency reported LD50 of diclofenac sodium in rats was 53-1500 mg/kg (European Agency for the evaluation 2003). However LD50 value give only information about animal mortality, but not indicator for other parameters of toxicity (Walum, 1998), so carrying out of other investigations such as hematological, pathological and genetotoxicity will give better toxicity profile.

TIND-7 exhibited no change in all observational parameters and the exception was the occurrence of dose-dependent increase in rate of respiration manifested as gasping of air and was predominantly in rats received dose of 2000 mg/kg. This revealed that one of expected side effect of TIND-7 is related to respiratory system. Respiratory depression is one cause of death in both experimental animals (Grinnell et. al., 2014) and human and related to use of many drugs such as morphine, which used heavily as analgesic for pain in many disease states.

Careful monitoring of the promising anti-inflammatory (TIND-7 entity) for this respiratory toxicity may be has worth value upon compared to morphine.

Finally, we conclude that, all the six compounds have pronounced anti-inflammatory activity and the TIND-7 was the most promising one with significant margin of safety comparable to dicolofenac. On the other hand, more studies are required to investigate the hematological, pathological and genetotoxicity to complete toxicity profile, whilst deep pharmacodynamics experiments should be conducted to elucidate the exact mechanism of action underlay the proved anti-inflammatory activity.

ACKNOWLEDGEMENT

This research is a part of a project entitled "The **O**mdurman-**A**imun-**T**ilal **P**roject for **D**rug **D**iscovery and **D**evelopment, 2015-2035 (**OAT-PDDD-2015-2035**)". This project was funded by the self-resources in a collaborative manner (No Grant Number was provided). The efforts of the collaborations are gratefully acknowledged.

Conflict of interests

The authors declare that they have no competing interest.

REFERENCES

1. Abdel-Aziz H. A, Elsaman T, Al-Rashood K. A. New 1, 2-and 1, 3 azalidesof amino-2-substituded-1H-isoindoles. Heteocyclic, 2014; 89: 995-1007.
2. Al-Qaisi J. A., Alhussainy T. M., Qinna N. A. Synthesis an pharmacological evaluation of aminoacetylenic isoindoline-1,3-dione derivatives as anti-inflammatory agents. Arabian journal of chemistry, 2014; 7: 1024-1030.
3. Armoiry X, G.Aulagner and T. Facon Lenalidomide in the treatment of multiple myeloma: A review. Journal of Clinical Pharmacy and Therapeutics, 2008; 33: 219–226.
4. Cong HH, Khaziakhmetova VN, Zigashina LE. Rat paw oedema modeling and NSAIDs: Timing of effects. Int J Risk Saf Med., 2015; 27: S76-77.
5. Derle D. V, Bele M, Kasliwal N. In vitro and in vivo evaluation of mefenamic acid and its complexes with β-cyclodextrin and HP- β-cyclodextrin. Asian journal of pharmaceutics, 2008; 30-34.
6. Dhar S, Seth J, Parikh D. Systemic Side Effects of Topical Corticosteroids. Indian Journal of Dermatology, 2014; 59: 460-464.
7. Dubois RN Role of Inflammation and Inflammatory Mediators in Colorectal Cancer. Transactions of the American Clinical and Climatological Association, 2014; 125: 358-373.
8. Farhangi MA, Keshavarz S-A, Eshraghian M, Ostadrahimi A, Saboor-Yaraghi A-A White Blood Cell Count in Women: Relation to Inflammatory Biomarkers, Haematological Profiles, Visceral Adiposity, and Other Cardiovascular Risk Factors. Journal of Health Population and Nutrition, 2013; 31: 58-64.
9. Ferenc Csende, Ferenc Miklós, and Andrea Porkoláb Design and synthesis of some isoindoline derivatives as analogues of the active anti-inflammatory Indoprofen. Arkivoc, 2013; 378-388.
10. Grinnell SG, Majumdar S, Narayan A. Pharmacologic Characterization in the Rat of a Potent Analgesic Lacking Respiratory Depression,

IBNtxA. The Journal of Pharmacology and Experimental Therapeutics, 2014; 350: 710-718.
11. Harirforoosh S, Asghar W, Jamali F. Adverse effects of non-steroidal anti-inflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Pharm Sci., 2013; 16: 821-47.
12. Klaus Speck and Thomas Magauer The chemistry of isoindole natural products. Beilstein J Org Chem., 2013; 9: 2048–2078.
13. Kumar A, Panghal S, Mallapur SS, Kumar M, Ram V, Singh BK Antiinflammatory Activity of Piper longum Fruit Oil. Indian Journal of Pharmaceutical Sciences, 2009; 71: 454-456.
14. Kumar S, Kumar N, Roy P. Synthesis anti-inflammatory, and anti-proliferative activity evaluation of isoindole, pyrrolopyrazine, benzamidazoisoindole, and benzamidazopyrrolopyrazine derivatives. Mol Divers, 2013; 17: 753.
15. Lam FW, Vijayan KV, Rumbaut RE Platelets and their interactions with other immune cells. Comprehensive Physiology, 2015; 5: 1265-1280.
16. Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. Nutr Rev., 2007; 65: S140-6.
17. Mansouri MT, Hemmati AA, Naghizadeh B, Mard SA, Rezaie A, Ghorbanzadeh B. A study of the mechanisms underlying the anti-inflammatory effect of ellagic acid in carrageenan-induced paw edema in rats. Indian Journal of Pharmacology, 2015; 47: 292-298.
18. Nath P, Yadav AK Acute and sub-acute oral toxicity assessment of the methanolic extract from leaves of Hibiscus rosa-sinensis L. in mice. Journal of Intercultural Ethnopharmacology, 2015; 4: 70-73.
19. Organisation for Economic Co-operation and Development (OECD) OECD guideline for testing chemicals. Acute Oral Toxicity-Up-and-Down-procedure, 2001; 1-26.
20. Posadas I, Bucci M, Roviezzo F. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. British Journal of Pharmacology, 2004; 142: 331-338.
21. Salvemini D. Wang Z. Wyatt P. S. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Bridsh Journal of Pharmacology, 1996; 118: 829-838.
22. The European Agency for the evaluation of medicinal product, Veterinary Medicine and Inspection, Committee for Veterinary Medicinal Products. Diclofenac summary report. EMEA/MRL/885/03-Final. Available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500013751&mid=WC0b01ac058008d7ad. Date of access November, 2003; 26: 2016.
23. Walum E. Acute oral toxicity. Environmental Health Perspectives, 1998; 106: 497-503.