In-vivo Anti-inflammatory, Analgesic and Anti-pyretic Activities of Synthetic Indole Derivatives in Mice

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Research Article

Keywords: Indoles, Indomethacin, Paracetamole, Albino mice, anti-inflammatory, analgesic, anti-pyretic

Posted Date: August 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1911507/v1

Abstract

Background

Various natural compounds have an aromatic heterocyclic compound “Indole” consists of bicyclic structure as a parent constituent in their composition that is used as an important precursor in many pharmaceutical industries and is widely accepted as a substance of fragrance to many novel compositions. In present study some synthetic indole derivatives are used to check their biological potential by performing anti-inflammatory, analgesic and anti-pyretic activity in albino mice.

Methods

Albino mice of either sex of reproductive age were used for each study and number of animals for each group in respective studies was kept 5. In anti-inflammatory activity, the Negative Control (NC) and Positive Control (PC) group animals were treated with normal saline and 10mg/kg of indomethacin respectively. The treated groups received the twenty four different synthetic chemicals, after 30 minutes of sub cutaneous injection of carrageenan. In analgesic activity, hot-plate method is used and for each group the latency period was recorded at zero moment of the provision of required dose and after 30, 60, 90, 120 and 180 minutes. In anti-pyretic activity, Pyrexia was induced by using Brewer's yeast method. Before any treatment and then after duration of 18 hours, the rectal temperatures were recorded. The animals, which showed the abnormally high rectal temperature, were selected for the further chemical investigation. SPSS.20 software is used for statistical analysis.

Results

This study helped to screen out the most potent indole derivative 3a-II and 4a-II from the 24 synthetic indole derivatives which demonstrated the best anti-inflammatory, analgesic and anti-pyretic potential as compared to remaining ones.

Conclusion

The above findings indicate that only two chemicals 3a-II (*N*-(4´-Chlorophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine) and 4a-II (*N*-(4´-Methylphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine) have given the desired effects like anti-inflammatory, analgesic and anti-pyretic.

Introduction

With the increase in human population, the need of higher yield and production of crops is mandatory for meeting the food requirements. For this purpose, several techniques have been developed. One strategy is based on the control of losses associated with the insects and pests. The use of poisonous chemicals is introduced in the field of agriculture for the control of the insects and pests. The excessive application of insecticides, pesticides and many other commercially produced chemicals is in practice now for the higher production of crops. These harmful chemicals ultimately enter into the food chain and cause

multiple physiological and anatomical aberrations in the dependent living organisms. The malignancies and the inflammatory reactions are the general outcome of toxicity of insecticides and pesticides. The topic of detoxification of these chemicals remained the topic of interest for the avoidance of such health damages [1].

The indoles are present naturally in some plants and fungi. These are among the most versatile and widely utilized nitrogen-based heterocyclic scaffolds. These are used in the synthesis of a wide range of synthetic organic molecules. Because of their biological and pharmacological properties, indole-based compounds are extremely important among the heterocyclic structures (2). Oxypertine, an indole derivative, is the antipsychotic and antidepressant, commonly used to treat schizophrenia (3). Indole 3-carbinol is a major bioactive component found in cruciferous vegetables. It has been studied for its potential to prevent a variety of malignancies (breast, prostate, colorectal, lymphoma and trans-placental cancer in offspring) (4, 5, 6). Considering their medicinal potential, the synthetic indole derivatives have been evaluated in the present study to check out their potential against pyrexia, analgesia and inflammatory diseases.

Methodology

Experimental animals and their maintenance:

Adult virgin albino mice of either sex weighing 30-32g of around 10-12 weeks of age were used in experimental work. These animals were kept in separate iron cages gauzed with stainless steel in animal house of Department of Zoology, University of Sargodha. All animals were provided the standard feed and water. Remaining food particles, excreta and any other debris were removed from cages on daily basis. Fresh paper cuttings were provided as bedding twice a week instead of wood shavings. The experimental animals were kept under a standard protocol of 12-12 hour dark - light cycle under ambient humidity (45%) and temperature (23±3ºC).

Dose regime and safety profile:

All 24 chemicals were tested at 10mg/kg concentrations to record analgesic, anti-pyretic or anti-inflammatory capacity through intra-gastric exposure. However, none of these chemicals have shown any sign of overt toxicity (frequent optic or oro-anal discharge, profuse urination, lethargy, abstained feeding and improper gait etc) or behavioral changes (aggression, restlessness etc) on 10mg/kg or lower intra-gastric exposures.

Carrageenan-induced paw edema:

Methodology:

All the test chemicals were administrated in a dose of 10mg/kg to the animals. After 30 minutes, the 0.01ml of 1% freshly prepared carrageenan solution was injected sub cutaneously in sub plantar region of right hind paw. The Negative Control (NC) and Positive Control (PC) group animals were treated with

normal saline and 10mg/kg of indomethacin respectively in place of test chemicals. The time taken for the paw edema to subside was recorded using a vernier caliper at different time intervals (20min, 40min, 60min…..200min) (7, 8).

Antipyretic activity:

Brewer's yeast induced Pyrexia:

Antipyretic activities of all chemicals were determined injecting 20 mg/kg of Brewer's yeast (20% suspension) following the method of Tesema and Makonnen (2015). Animals showing an increase in rectal temperature from 0.3 to 0.6°C were used for the further investigation. These selected animals (n: 5) were distributed in 26 groups. Twenty four groups received one of the 24 chemicals to be tested (10mg/kg) while one of the remaining two groups received Paracetamol (150mg/kg) and the second was given vehicle (1% aqueous DMSO) only. The rectal temperature was recorded by K-type thermocouple for 3 consecutive hours on hourly basis after respective dose administration with the help of digital thermocouple (K-type)

Analgesic Activity (Hot plate method):

Evaluation of analgesic capacity of the novel chemicals was performed using the temperature tolerance ability of the animals in each experimental group by placing them one by one in a restrainer on the hot-plate maintained at 55°C. The time taken for first reaction to the heat by each animal was recorded. Individual sensitivity responses include jumping, withdrawal and licking of the paws etc. The time period (latency period) between the moments an animal first placed in the restrainer and the moment it showed any of the above response ware recorded by the digital stopwatch. For each group the latency period was recorded at zero moment of the provision of required dose and after 30, 60, 90, 120 and 180 minutes. These data obtained in treated groups were compared statistically with standard drug (indomethacin 10mg/kg) and NC (1% aqueous DMSO) group for significance of difference (9, 10).

Data analysis and statistical applications:

Data obtained was analyzed through SPSS.20 software, ANOVA and Tukey’s Multiple Range Test.

Results

General responses to the anti-inflammatory, analgesic and anti-pyretic activities of the individual chemicals are enumerated in the table below.

	Code	IUPAC Names of the Compounds	Anti-inflammatory activity	Analgesic activity	Anti-pyretic activity
1	349	4,6-Dimethoxy-2,3-diphenyl-1*H*-indole	×	×	×
2	364	4,6-Dimethoxy-2,3-diphenyl-1*H*-indole-7-carbaldehyde	×	×	×
3	382	*N*-Phenyl (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	×	×	×
4	384	*N*-(3′-Chlorophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	×	×	×
5	4a-ll	*N*-(4′-Methylphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	□□	A□□	P□□
6	385	*N*-(2′,3′-Dichlorophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	×	×	×
7	3a-ll	*N*-(4′-Chlorophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	□□	A□□	P□□
8	387	*N*-(3′-Methylphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	×	×	×
9	388	*N*-(2′,3′-Dimethylphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	×	×	×
10	389	*N*-(4′-Methoxyphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	¥	¶	ð
11	390	*N*-(3′,4′,5′-Trimethoxyphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	×	×	×
12	392	*N*-(3′-Nitrophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine	¥	¶	ð
13	21-a	4,5,6-Trimethoxy-2,3-diphenyl-7-phenylaminomethyl-1*H*-indole	×	×	×
14	21-b	4,5,6-Trimethoxy-7-(3′-methylphenylaminomethyl)-2,3-diphenyl-1*H*-indole	×	×	×
15	21-c	4,5,6-Trimethoxy-7-(4′-methylphenylaminomethyl)-2,3-diphenyl-1*H*-indole	×	×	×
16	21-d	4,5,6-Trimethoxy-7-(4′-methoxyphenylaminomethyl)-2,3-diphenyl-1*H*-indole	×	×	×
17	21-e	7-(3′-Chlorophenylaminomethyl)-4,5,6-trimethoxy-2,3-diphenyl-1*H*-indole	×	×	×
18	21-f	7-(4′-Chlorophenylaminomethyl)-4,5,6-	×	×	×

		trimethoxy-2,3-diphenyl-1*H*-indole			
19	21-g	7-(3´,4´-Dichlorophenylaminomethyl)-4,5,6-trimethoxy-2,3-diphenyl-1*H*-indole	×	×	×
20	21-h	4,5,6-Trimethoxy-7-(3´-nitrophenylaminomethyl)-2,3-diphenyl-1*H*-indole	×	×	×
21	21-i	4,5,6-Trimethoxy-7-(4´-nitrophenylaminomethyl)-2,3-diphenyl-1*H*-indole	×	×	×
22	21-j	7-(3´-Bromophenylaminomethyl)-4,5,6-trimethoxy-2,3-diphenyl-1*H*-indole	×	×	×
23	21-k	7-(4´-Bromophenylaminomethyl)-4,5,6-trimethoxy-2,3-diphenyl-1*H*-indole	×	×	×
24	21-L	4,5,6-Trimethoxy-7-(3´,4´-dimethylphenylaminomethyl)-2,3-diphenyl-1*H*-indole	×	×	×

Table.1 **n=5 (**five animals were used for each above mentioned activity), ×: no activity, **¥**: pro-inflammatory, **¶**: pain intensifier (anti-analgesic), **ð**: pyretic (increases body temperature), **▯▯:** anti-inflammatory, **A▯▯:** analgesic, **P▯▯:** anti-pyretic

There are twenty four synthetic indole derivatives which were used in this study to check their anti-inflammatory, analgesic and anti-pyretic potential by using different appropriate methods for each activity in albino mice. From this list of chemicals, majority of derivatives have shown null potential in all of three activities, 2 chemicals 389(*N*-(4´-Methoxyphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine) and 392(*N*-(3´-Nitrophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine) have shown pyretic, anti-analgesic and inflammatory effects and only two chemicals 3a-II(*N*-(4´-Chlorophenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine) and 4a-II(*N*-(4´-Methylphenyl) (4,6-dimethoxy-2,3-diphenyl-1*H*-indol-7-yl)methanimine)have given the desired effects like anti-inflammatory, analgesic and anti-pyretic.

Carrageenan induced paw edema in mice

As shown in the table below administration of 10mg/kg of both the 3a-II and 4a-II and the indomethacin (10mg/kg) have prominently reduced paw edema with in 60min after the first injection as compared to the NC group which took 80min for the paw edema to subside.

Sr.no	Chemical code:	Effect	Time to subside paw edema
1	**Control**	Nil	180min
2	**Indomethacin**	Anti-inflammatory	90min
3	**3a-II**	Anti-inflammatory	80min
4	**4a-II**	Anti-inflammatory	85min

Table:2-Effect of test chemicals on carrageenan-induced paw edema

Anti-pyretic activity:

The two chemicals (3a-II and 4a-II) shown antipyretic activity almost parallel to Paracetamol as enumerated in the table below

Sr. No	Groups	Before yeast	After 18hr	Temperature after treatment		
				1hr	2hr	3hr
1	Control	37.88 ± 0.1^{a}	$38.5\pm.1^{a}$	$38.2\pm.02^{a}$	$38.4\pm.1^{b}$	$38.4\pm.03^{c}$
2	Paracetamol	$37.97\pm.1^{a}$	$38.6\pm.01^{a}$	$38.3\pm.1^{a}$	$38.1\pm.1^{a}$	$38\pm.02^{b}$
3	3a-II	$37.9\pm.1^{a}$	$38.5\pm.1^{a}$	$38.2\pm.1^{a}$	$38.1\pm.03^{a}$	$38\pm.02^{ab}$
4	4a-II	$37.8\pm.4^{a}$	$38.5\pm.1^{a}$	$38.3\pm.1^{a}$	$38.2\pm.02^{a}$	$38.1\pm.02^{b}$

Table: 3-Statistical analysis (ANOVA: two factors), group means ±SEM,$^{a\ b\ c\ d}$: Anyone two groups not sharing a lower case letters differ significantly from each other

Analgesic activity:

As shown in the table administration of indomethacin (10mg/kg) has significantly extended the time threshold at 60 and 90min but after 90min there is found the same trend of decline in time threshold like all other groups control, 3a-II and 4a-II chemicals at 30, 60, 90,120 and 180min by comparing them with 0min (standard timing).

The results of analgesic activity are shown in following table

Sr.no	Groups	0 min	30 min	60 min	90 min	120 min	180 min
1	Control	5.67±.57[a]	5.23±.68[a]	4.74±.34[a]	4.37±.47[a]	4.16±.62[a]	4.02±.54[a]
2	Indomethacin	5.97±.84[a]	5.92±.46[a]	6.44±.08[a]	6.81±.75[a]	5.76±.87[a]	5.59±.70[a]
3	3a-II	8.33±.57[a]	8.33±.57[a]	6.67±.57[a]	6±.00[a]	6±.00[a]	5.67±.57[a]
4	4a-II	6.33±.57[a]	6±.00[a]	6±.57[a]	5.33±.57[a]	4.67±.57[a]	4±.00[a]

Table:4- Data is expressed as mean ±SD of n=5. *P□0.05 compared with control. SD: Standard deviation

*0min: initial response of animals before administration of any treatment (NT) No treatment;*30, 60,…..180min: response of animal after 30, 60,….180min of administration of treatment

Discussion

The analgesic activity is performed by hot plate method in which pain is induced that generates the reflexes at the spinal level and usually these pain reflexes are controlled by brainstem and cortical portion of brain (11). In hot plate method thermal heat is used to induce pain in the specimen to note the behavior like jumping, withdrawal and licking of paws etc. This hot plate method is used to induce acute pains by heat mediated damage of tissues which releases peripheral mediators (12, 13, 14).

Anti-inflammatory activity is performed by using λ-carrageenan. The mechanism of inflammation caused by λ-carrageenan is well characterized both at the cellular and molecular level as in this method inflammation occurs in two phases. In first phase, various inflammatory mediators like serotonin, histamine and bradykinin are released and in second phase cytokines as PGs, IL-6, IL-1 β and TNF-α are generated (15). The level of COX-2 which causes the production of PGs (prostaglandins) in response to inflammation is shown maximum in the paw edema at the late phase (16). The probable reason for those two chemicals 3a-II and 4a-II which have shown anti-inflammatory effect is that they may be lowers the level of inflammatory mediators at the site of inflammation.

Those agents or compounds which have the potential to lower the body temperature are known as antipyretics. Fever is usually caused by some disease, infection, inflammation or any type of tissue damage (17) and body requires a delicate balance of temperature for its regulation for which mainly hypothalamus helps to regulate the body temperature by maintaining a set point. Exogenously, NSAIDs are important to regulate the body temperature or giving a relief from fever by blocking the COX-2 production of prostaglandins (PGs) (18, 19). In present study it has been seen that exposure to 3a-II and 4a-II indoles reduces the body temperature of specimen that is comparable to standard drug Paracetamol.

Conclusion

On the basis of current observations, it can be concluded that both chemicals 3a-II and 4a-II play protective role against λ-carrageenan induced paw edema. Temperature tolerance ability was also improved due to their use. Brewer's yeast induced pyrexia based observations indicate the antipyretic activity of these two chemicals. These findings point out their importance as anti-inflammatory, analgesic and anti-pyretic compounds. The proposed mechanism of these indoles might be due to the inhibition of PGs by suppressing the level of inflammatory mediators that are the major cause of symptoms of inflammation (swelling, pyrexia, pain, edema accumulation etc).

Declarations

Ethics approval and consent to participate

All experimental procedures complied with the National Institute of Health Guide for the Care and Use of Laboratory Animals (USA) and approved by the Ethical review committee of Department of Zoology, University of Sargodha.

We confirm that our study was performed in accordance with the Arrive guidelines. All the methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

There is no funding for this study.

Authors' contributions

Khawaja Raees Ahmad conceived and designed the experiments. Saira Siddique, Rabiyah Ali performed the experiments. Khawaja Raees Ahmad, Sadia Suleman and Syeda Nadia Ahmad analyzed the data. Saira Siddique and Syed Kashif Nawaz wrote the manuscript. All authors read and approved the final manuscript

Acknowledgement:

We thank the University of Sargodha, Sargodha, Pakistan for permission to conduct this study and provision of appropriate funding. We also thank the lab and animal house facilities for their participation in this research work.

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