Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INVOLVEMENT OF OPIOIDERGIC, TRYPTAMINERGIC AND K^+_{ATP} CHANNELS IN THE ANTINOCICEPTIVE ACTION OF INDAZOLE AND ITS DERIVATIVES

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ABSTRACT

The present study was designed to investigate the anti-nociceptive action of indazole, 5 – aminoindazole and 6 – nitroindazole and the possible mechanisms involved in this effect. The anti - nociceptive effect was studied using three different nociceptive assays. These are acetic acid assay, formalin induced nociception and hot plate methods. Swiss albino mice were selected for this study. The participation of opioid, tryptaminergic, dopaminergic and K^+_{ATP} channels in the anti-nociceptive effect were also studied by using appropriate interacting agents. Indazole and its derivatives exhibited a significant dose dependent inhibition of acetic acid writhing. The paw licking response time was reduced in formalin induced nociception in a dose dependent manner by indazole and its derivatives. A significant increase in withdrawal latency time was also observed in thermal nociception after indazoles treatment. These observations revealed the anti-nociceptive effect of indazole and its derivatives. The participation of opioid system and K^+_{ATP} channel was identified in the anti - nociceptive effect of indazole and its derivatives. Additionally, participation of tryptaminergic 5 - HT_3 receptors could also be recorded in the anti-nociceptive action of 6 – nitroindazole, but not in other investigated compounds. Pretreatment with haloperidol, a non specific dopamine receptor antagonist potentiated the anti - nociceptive response of indazole and its derivatives. The present study identified the anti - nociceptive effect of indazole and its derivatives in mice and mechanisms involved in the anti - nociceptive activity of these compounds.

KEY WORDS: **Indazoles, Antinociceptive effect, Neuronal mechanisms**

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INTRODUCTION

Many of the currently available analgesic drugs though offer a near satisfactory relief from pain are invariably accompanied by intolerable side effects on routine use. Apart from NSAID and opiate variants, new molecules designed specifically as analgesic agents have not been forthcoming in spite of the obvious need. Hence many attempts are made worldwide to develop novel analgesic compounds with fewer side effects. Indazoles are emerging as pharmaceuticals with a specific use in certain diseases. Investigations carried out in recent decades have identified the potential usefulness of these compounds in several biological conditions such as inhibition of apoptosis[1], treatment of rheumatoid arthritis[2], anti-proliferative activity[3], treatment of hypertension[4], anti psychotic activity[5], hypotensive activity[6], treatment of obesity[7], tumor cell cytotoxic assays[8], anti - hyperlipidemic activity[9], trichomonacidal activity[10], Analgesic and antipyretic activity[11] and anti-inflammatory activity[12]. The above interesting biological activities of indazole derivatives kindled an interest whether indazole and derivative could be a potential source for antinociceptive drugs. Hence, in the present study, indazole and derivatives of indazole viz. indazole, 5 – aminoindazole, 6 – nitroindazole were selected and screened for the anti-nociceptive activity. The study was extended to investigate the mechanisms underlying the anti-nociceptive activity of the test compounds.

MATERIALS AND METHODS

Animals

Adult Swiss albino mice of either sex weighing 25 – 30g were used in the present study and these were purchased from the Kings Institute of preventive medicine, Gundy. The animals had free access to food and water and maintained at 24 ± 1° C temperature with 12h day/ 12h night cycle. All the experiments were carried out between 09.00 and 13.00 hours to avoid circadian variation. The experiments were carried out in Sree Balaji Medical College and Hospital, Chennai. The experimental protocol was approved by the institutional animal ethical committee (002/02/IAEC/2014/SBMCH). In all the experimental studies each group consisted of six animals.

Drugs and chemicals

After careful scrutiny of the literature, indazole, 5 – aminoindazole and 6 – nitroindazole were selected for the study (Fig- 1) and these were purchased from Sigma Aldrich, USA. The test compounds were prepared as a fine suspension in 0.5% carboxy methyl cellulose (CMC) and injected i.p in doses ranging from 12.5 - 100 mg/kg, 30 min prior to the testing procedures. Morphine sulphate (Varma Pharmaceuticals, Chennai) and Diclofenac were used as standard drugs for comparison. Other interacting chemicals used were naloxone hydrochloride (Endo labs, USA), haloperidol (Sigma Chemical Co, USA), glibenclamide (Dr. Reddy's Laboratory, India) and ondansetron (Sunvet Health Care, India).

Acute toxicity study

Acute toxicity study was carried out according to guidelines 423 of organization for economic cooperation and development (OECD) [13]. A group of three mice received test compounds in a dose of 2 g/kg by i.p route and they were monitored continuously for 6 h to observe any changes in autonomic and behavioral responses and kept under observation for a period of two weeks (14 days) to detect any mortality.

Evaluation of locomotor activity[14]

The effect of various indazoles on locomotor activity was tested using an open field apparatus. The apparatus consists of a wooden box (96 x 96 x 30 cm), where the arena is divided in to 16 equal squares. The number of squares crossed by mice with all the paws was counted in a 5 min session. The animals were treated with test compounds in a dose of 100 mg/kg, i.p or vehicle (0.5% CMC) 30 min prior to the testing procedure.

Rota rod test[15]

The motor co – ordination of mice was tested on a rota – rod apparatus (15 revolutions per minute) after treatment with indazole and its derivatives in a dose of 100mg/kg, i.p. The ability of the animal to remain on the rotating rod was measured by recording the balancing time and compared with vehicle treated animals. The cut off time employed in this method was 60 sec.

Assessment of anti-nociceptive action

Acetic acid induced abdominal constriction assay[16]

Acetic acid (0.6%, 10 ml/kg) was administered i.p and the number of abdominal constrictions was counted during the following 15 min period. A significant reduction in the number of abdominal constrictions after any drug treatment when compared with vehicle treated animals was considered as antinociceptive response. Different doses (12.5 – 100 mg/kg, i.p) of indazole and its derivatives were administered 30 min prior to acetic challenge. A separate group of mice received morphine 5 mg/kg i.p 30 min prior to acetic acid injection. The percentage inhibition of writhings compared to vehicle treatment was calculated using the formula (C-T/C) x 100, where C is the number of abdominal constrictions recorded in vehicle treated animals and T is the number of abdominal constrictions in the treatment group.

Formalin assay[17]

Each mouse was placed in an observation chamber, 5 min prior to the formalin injection for acclimatization to the new environment. 1% formalin (50 µL) was administered s.c in to the plantar surface of the left hind paw and the time spent in licking/biting the injected paw was recorded for a period of 30 min. The early phase of nociceptive response normally peaks from 0-10 min and late phase from 10-30 min after formalin injection. Indazole and its derivatives were administered i.p in doses of 12.5, 25, 50 or 100 mg/kg, 30 min before the testing procedure. Morphine 5 mg/kg was administered i.p 30 min prior to formalin an injection in a separate group of mice. The percentage inhibition of paw licking response time in all treatment groups was calculated using the formula (C-T /C) x 100, where C is the paw licking response time observed in control animals and

T is the paw licking response time in the treatment group.

Hot plate test[18]

The central analgesic activity of indazole and its derivatives was assessed using the hot plate test. Before conducting the study, the Swiss albino mice were screened by sensitivity tests by placing the animals on the hot plate. Any animal that withdrew its hind paw or jumped in response in 5 seconds were rejected from the study. The animals were individually placed on a hotplate which was maintained at a constant temperature (52 ± 1°C). The latency to the first sign of paw licking or jump response to avoid heat nociception was taken as an index of the nociceptive threshold with a cut off time of 30 sec. Indazole and its derivatives in a dose range of 12.5 - 100 mg/kg were injected i.p 30 min before the testing procedure. A group of mice that received morphine (10 mg/kg, i.p) 30 min prior to the test procedure was used for comparison. The analgesic response was expressed as % maximum protective effect (MPE), which was calculated using a formula

$$\% \text{ MPE} = [(\text{Test latency-control latency})/(\text{cut off time-control latency})] \times 100$$

Investigation on mechanisms

A dose of indazole and its derivatives which produced nearly 50% inhibition of acetic acid induced nociception was selected to study the various mechanisms involved in the antinociceptive effect. The following doses of the test compounds were selected for this purpose; indazole 50 mg/kg, 5 – aminoindazole 12.5 mg/kg, 6 - nitroindazole 50 mg/kg. To explore the possible mechanisms involved in the anti-nociceptive action of test compounds, mice were pretreated (i.p) with the following interacting chemicals 15 min prior to the administration of test compounds and subjected to acetic acid assay after 30 min.

(a) Opioid antagonist naloxone 5 mg/kg[19].
(b) 5-HT_3 receptor antagonist ondansetron 0.5 mg/kg[20].
(d) Dopaminergic receptor antagonist Haloperidol 1 mg/kg[21].
(e) K^+_{ATP} blocker glibenclamide 10 mg/kg[22].

Statistical analysis

The results were subjected to one way analysis of variance (ANOVA) followed by Dunnett's t test, unpaired and paired 't' test utilising SPSS 16 software and a p value less than 0.05 was considered for statistical significance.

RESULTS

Acute toxicity testing

The test compounds, indazole and its derivatives even in a dose of 2 g/kg did not produce any significant change in the behavioral or autonomic responses in mice compared to vehicle treatment. Continuous monitoring of these animals for another two weeks did not reveal any mortality.

Motor performance and locomotor activity

Treatment with indazole and its derivatives in a dose of 100 mg/kg, did not alter the balancing time of mice on a rotarod or the number of squares crossed in the open field apparatus when compared to vehicle treated animals (data not shown).

Acetic acid induced abdominal constriction assay

The mean number of abdominal constrictions in vehicle treated control animals was 34.16 ± 0.46. Morphine treatment almost abolished the abdominal constrictions after acetic acid injection in mice (Table- 1) thus producing 97.11% inhibition of the nociceptive behavior. A significant reduction in the number of abdominal writhings was observed after treatment with different doses of indazole and its derivatives (Table- 1). 5 – aminoindazole exhibited maximum inhibition of nociception (100%) in a dose of 100 mg/kg. In a similar dose the maximum inhibition observed for the other tested indazole derivatives was around 90% (Table- 1). The effective dose of various indazoles that produced 50% inhibition of nociception in acetic acid writhing (ED_{50}) was calculated by plotting a dose response curve (not shown). The ED_{50} values of the test compounds in increasing order are of 5 - aminoindazole (12.5 mg/kg), Indazole (50 mg/kg) and 6 - nitroindazole (50 mg/kg).

Formalin nociception

In vehicle treated control group the paw licking response time was 56.83 ± 1.26 sec in acute phase and 70.33 ± 2.23 sec in chronic phase (Table- 2). Morphine in a dose of 5 mg/kg significantly inhibited the paw licking response time in both acute and chronic phases of nociception (Table- 2). Thus morphine produced 87.10% inhibition of acute and 97.39% inhibition of chronic phases of formalin induced nociception. Indazole and its derivatives significantly inhibited the paw licking response time in acute phase of formalin nociception in doses of 25, 50 or 100 mg/kg (Table- 2). However, a significant reduction in the paw licking response time was noted in all the doses of indazole and its derivatives in chronic phase of formalin nociception (Table- 2). In both phases of formalin induced nociception, the maximum reduction in paw licking response time was exhibited by 5 – aminoindazole in a dose of 100 mg/kg and thus produced 65.98% inhibition of acute phase and 100% inhibition of chronic phase (Table- 2). Other tested compounds produced a significant reduction in paw licking response time in both acute and chronic phases of formalin nociception when compared to the vehicle treated group (Table- 2). In general, the inhibition of paw licking response exhibited by indazole and its derivatives was greater in the chronic phase of formalin nociception than in the acute phase.

Hot plate test

Treatment with morphine in a dose of 10 mg/kg produced a significant increase in reaction time of 28.33 ± 0.29 sec and offered 92.52% protection against the thermal nociceptive (Table- 3). Similarly, a significant increase in mean reaction time was observed after treatment with indazole and its derivatives (Table- 3).

The maximal protective effect offered by indazole (100 mg/kg) against thermal nociception was 47.76%, while that of other tested compounds ranged between 78.33 and 100.00% (Table- 3). 5 - aminoindazole produced a maximal protective effect (100%) against thermal nociception when compared to indazole (47.76%), 6 – nitroindazole (78.33%) or morphine (92.52).

Analysis of possible mechanisms of action of indazole and its derivatives

Indazole and its derivatives in selected doses showed a significant reduction in the number of abdominal constrictions in mice (Fig- 2). However, pretreatment with naloxone significantly attenuated the reduction in the number of abdominal constrictions produced by indazole and its derivatives. Pretreatment with ondansetron in vehicle treated group did not modify the number of abdominal constrictions in mice. However, ondansetron pretreatment significantly attenuated the reduction in the number of abdominal constrictions produced by 6 – nitroindazole alone without altering the response to other tested compounds (Fig- 3). The haloperidol *per se* pretreatment did not potentiate the number of abdominal constrictions when compared to vehicle treated group. However, the reduction in the number of abdominal constrictions elicited after indazole and its derivatives treatment was further potentiated by haloperidol pretreatment (Fig- 4). Glibenclamide *per se* did not alter the number of abdominal constrictions in mice compared to vehicle treatment. The decrease in the number of abdominal constrictions produced by treatment with indazole, 5 - aminoindazole and 6 - nitroindazole was significantly reversed by glibenclamide (Fig- 5).

Table 1

Effect of indazole and its derivatives on acetic acid – induced abdominal constrictions in mice

Dose of test compounds (mg/kg, i.p.)	Number of abdominal constrictions		
	Indazole	5 - Aminoindazole	6 - Nitroindazole
12.5	33.66 ± 0.21 (2.88)	20.16 ± 1.29[a] (41.83)	35.66 ± 1.21 (00.00)
25	24.83 ± 1.16[a] (28.36)	11.83 ± 0.88[a] (65.86)	29.50 ± 0.69[a] (14.88)
50	17.83 ± 1.23[a] (48.55)	4.66 ± 0.21[a] (86.55)	18.33 ± 0.61[a] (47.11)
100	2.50 ± 1.19[a] (92.78)	0.00 ± 0.00[a] (100.00)	3.16 ± 0.64[a] (90.88)

Each value represents the mean ± S.E.M of six observations.
The values in parenthesis indicate the percentage inhibition of abdominal constrictions.
The number of abdominal constrictions in vehicle treated animals was 34.16 ± 0.46.
The number of abdominal constrictions after morphine (5mg/kg) treatment was 1.00 ± 0.25[a] (97.11%).
[a]P < 0.05, compared with vehicle treatment.

Table 2

Effect of indazole and its derivatives on formalin – induced nociception (acute and chronic phases)

Dose of test compounds mg/kg, i.p.	Indazole		5 – Aminoindazole		6 – Nitroindazole	
	Acute	Chronic	Acute	Chronic	Acute	Chronic
12.5	56.83 ± 0.16 (0.00)	40.33 ± 1.21[a] (42.65)	56.83 ± 1.16 (0.00)	29.66 ± 1.78[a] (57.82)	56.33 ± 3.61 (0.87)	42.33 ± 1.12[a] (39.81)
25	54.50 ± 2.26[a] (4.09)	28.33 ± 1.83[a] (59.71)	49.66 ± 2.75[a] (12.61)	20.50 ± 3.13[a] (70.85)	55.66 ± 1.58[a] (2.05)	30.33 ± 2.17[a] (56.87)
50	45.16 ± 1.66[a] (20.53)	9.83 ± 3.24[a] (86.02)	40.50 ± 1.78[a] (28.73)	9.16 ± 2.18[a] (86.97)	48.83 ± 1.03[a] (14.07)	14.66 ± 2.04[a] (79.15)
100	38.66 ± 2.68[a] (31.97)	1.50 ± 0.22[a] (97.86)	19.33 ± 2.16[a] (65.98)	00.00 ± 0.00[a] (100.00)	41.83 ± 2.07[a] (26.39)	3.83 ± 2.69[a] (94.55)

Each value represents the mean ± S.E.M of six observations.
The values in parenthesis indicate the percentage inhibition of formalin – induced nociception.
The paw licking response times in vehicle treated animals were 56.83 ± 1.26 sec (87.10%) in the acute phase and 70.33 ± 2.23 sec (97.39%) in chronic phase.
The paw licking response time after morphine (5mg/kg) treatment was 7.33 ± 0.21 sec[a] in the acute phase (87.10%) and 1.83 ± 0.16 sec[a] (97.39%) in the chronic phase
[a]P < 0.05, compared with vehicle treatment.

Table 3
Effect of indazole and its derivatives on hot plate test in mice

Dose of test compounds (mg/kg, i.p)	Mean increase in reaction time (seconds)		
	Indazole	5 - Aminoindazole	6 - Nitroindazole
12.5	7.50 ± 0.22 (0.00)	12.33 ± 0.41[a] (20.90)	10.66 ± 1.31 (13.42)
25	10.00 ± 1.80 (10.47)	17.33 ± 1.21[a] (43.28)	15.16 ± 2.39[a] (33.57)
50	14.66 ± 1.21[a] (31.33)	26.16 ± 1.41[a] (82.81)	20.66 ± 1.66[a] (58.19)
100	18.33 ± 1.36[a] (47.76)	30.00 ± 0.00[a] (100.00)	25.16 ± 0.77[a] (78.33)

Each value represents the mean ± S.E.M of six observations.
The values in parenthesis indicate the percentage of maximal protective effect.
The mean increase in reaction time in vehicle treated animals was 7.66 ± 0.21 sec.
The mean increase in reaction time after morphine (10mg/kg) treatment was 28.33 ± 0.29 sec[a] (92.52%).
[a]P < 0.05, compared with vehicle treatment.

Figure 1
Structure of indazole and its derivatives

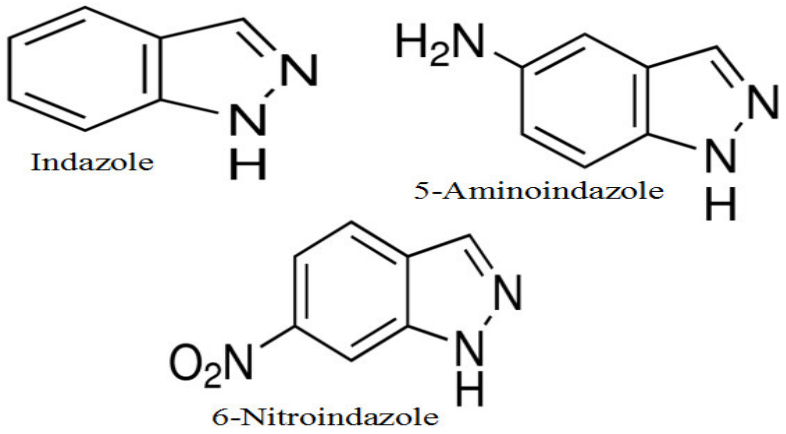


Figure 2
Effect of naloxone on indazole and its derivatives - induced inhibition of acetic acid writhing in mice. Each column represents the mean ± S.E.M. of 6 mice. *P < 0.05 when compared without naloxone

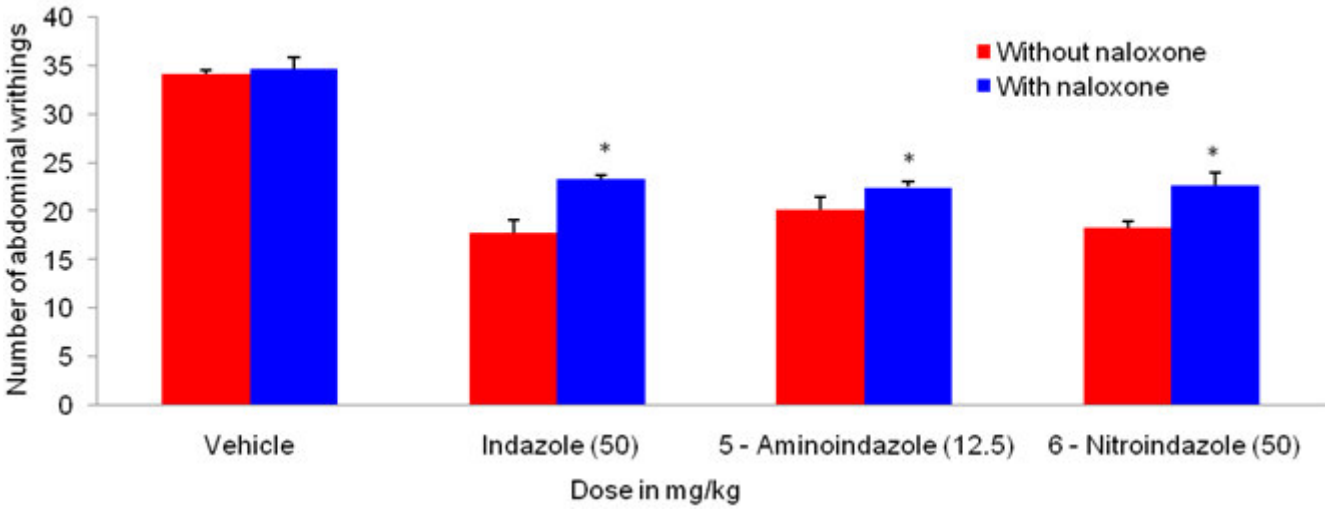


Figure 3

Effect of ondansetron on indazole and its derivatives - induced inhibition of acetic acid writhing in mice. Each column represents the mean ± S.E.M. of 6 mice. *P < 0.05 when compared without ondansetron.

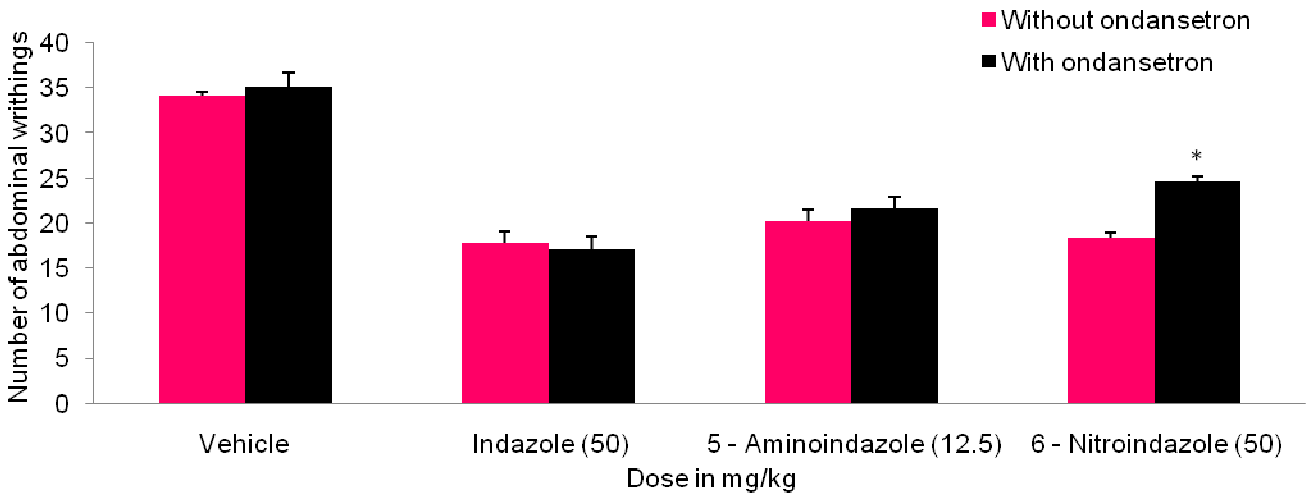


Figure 4

Effect of haloperidol on indazole and its derivatives - induced inhibition of acetic acid writhing in mice. Each column represents the mean ± S.E.M. of 6 mice. *P < 0.05 when compared without haloperidol.

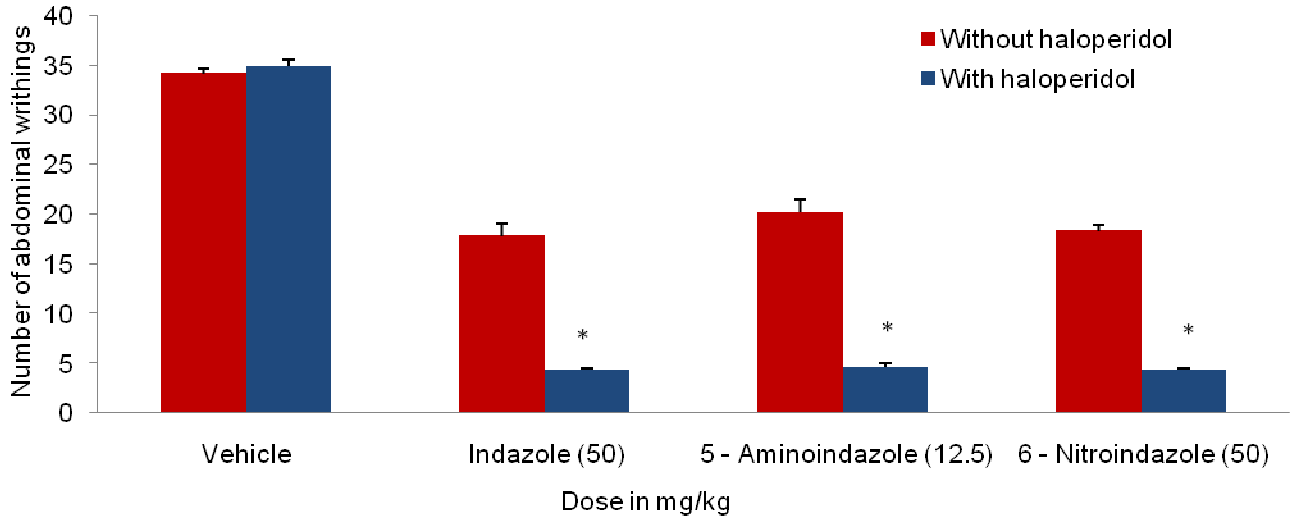
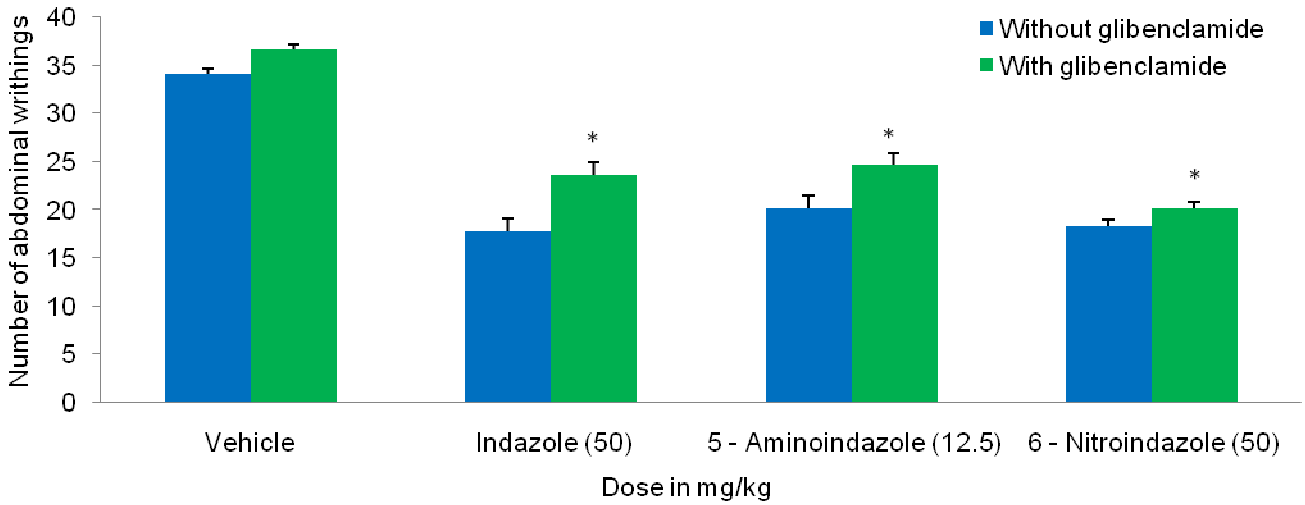


Figure 5

Effect of glibenclamide on indazole and its derivatives - induced inhibition of acetic acid writhing in mice. Each column represents the mean ± S.E.M. of 6 mice. *P < 0.05 when compared without glibenclamide.



DISCUSSION

In the present study, we demonstrated that systemic administration of indazole and its derivatives viz; 5 – aminoindazole and 6 - nitroindazole at the doses of 12.5, 25, 50 and 100 mg/kg evoked significant dose dependent inhibition against chemical and thermal induced nociception test models. Another important additional finding of the present study was the demonstration of the possible involvement of the opioid system, tryptaminergic system and ATP - sensitive K^+ channel pathway in the indazole and its derivatives induced antinociception in mice. The test compounds did not produce any mortality in mice even in a dose of 2 g/kg and hence may be considered as relatively safe. The present results also revealed that, systemic administration of indazoles in a dose of 100 mg/kg in mice did not produce any change in motor coordination or alteration in locomotor activity. These tests help us to rule out false positive results in anti-nociceptive response evaluated in different models of nociception. In order to evaluate the anti-nociceptive property of any new substance using behavioral nociceptive tests, it is considered necessary to employ different tests which differ in stimulus quality, intensity and duration[23]. Acetic acid writhing method[16] is commonly used to evaluate the anti-nociceptive activity which is mediated by inflammation[24]. The pain sensation is believed due to stimulation of peripheral nociceptive neurons by the released inflammatory mediators[25] and activation by non-selective cation channels located in the primary sensory pathways[26]. This nociceptive impulse is effectively suppressed by both opioids and non-steroidal anti-inflammatory agents. The investigational compounds, indazole and its derivatives showed a significant reduction in acetic acid induced abdominal constrictions in mice indicating that, these compounds may effectively suppress inflammatory and visceral pain (Table- 1). Among the tested compounds 5 – aminoindazole showed maximum inhibition of acetic acid induced abdominal constrictions in a dose of 100 mg/kg and thus it exhibited 100.00% inhibition of acetic acid induced nociception. In the present study formalin induced nociception was also employed to investigate the antinociceptive action of indazole and its derivatives. This method measures the ability of a substance to attenuate moderate continuous pain generated by injured tissue. Thus it differs from other traditional tests of nociception, which depend upon brief stimuli of threshold intensity where the nociceptive experience is short lasting[27]. Intraplantar injection of formalin results in licking and biting of the injected paw. This nociceptive behaviour appears in two distinct phases. The first phase starts immediately after injection of formalin and lasts for about five minutes. It is probably due to direct chemical stimulation of nociceptors[17]. This is followed by a quiescent period of about 10 minutes. The second phase starts approximately 10-15 minutes after formalin injection and may extend for 15-30 minutes. The second phase is considered to be due to a combination of an inflammatory reaction in the peripheral tissue and changes in central processing[27]. This nociceptive method is considered to indicate the ability of a substance to alleviate moderate continuous pain that is generated in injured tissue[27] and better correlations were suggested with clinical pain[28]. This method has been employed to investigate a variety of compounds, including opioids, NSAID and monoamines[29, 30, 31]. Hence, in the present study formalin test was also included to assess the antinociceptive activity of indazole and its derivatives. The results obtained from the formalin induced nociceptive method also confirmed the potent anti - nociceptive effect of indazole and its derivatives. In acute phase of formalin induced nociception, the maximum inhibition is produced by 5 – aminoindazole (65.98%) when compared to other tested compounds and they produced a lesser degree of inhibition ranging from 26% to 31% (Table- 2). Very potent inhibition of the inflammatory phase of formalin nociception was recorded for indazole and its derivatives. Administration of indazole, 5 – aminoindazole and 6 – nitroindazole resulted in more

than 94% inhibition of the late phase of formalin nociception was achieved (Table- 2). The present results further validate the anti-nociceptive potential of indazole and its derivatives in both neurogenic and inflammatory pain. However, a higher degree of inhibition of the late phase of formalin nociception by the tested compounds suggests better efficacy of these compounds against inflammatory pain. The hotplate method was preferred in this study because all the four limbs and even the tail of the animal are stimulated simultaneously[32]. Such heterotopic stimuli involving large body areas undoubtedly trigger diffuse noxious inhibitory controls with supraspinal origins[33, 34] and only very potent analgesic drugs exhibit good anti-nociceptive effect in this test procedure. A significant increase in maximal protective effect was observed after treatment with indazole and its derivatives (Table-3). The maximal protective effect observed for indazole was 47.76% whereas other compounds produced a higher degree of protection and the effect of 5 – aminoindazole being the maximum (100.00%) which is higher than the morphine protection (92.52%). The results obtained in the hot plate assay indicate the efficacy of these compounds on thermal nociception. The results obtained from the above three procedures which employed different types of nociception indicate the anti-nociceptive potential of these compounds against pain of different origin. Pain transmission is a complex process that involves multiple neuronal mechanisms and ion channels. They initiate and transmit the pain signals along the pain pathway. The present study analyzed some of these possibilities in the anti - nociceptive effect of indazole and its derivatives by employing suitable interacting drugs. Activation of the opioidergic system produces potent analgesia.The results of the present study clearly indicates that an opioid antagonist naloxone completely reversed the anti-nociceptive effect of the investigated indazole and its derivatives (Fig- 2). This observation provides conclusive evidence for the role of opioid mediated anti-nociceptive action of indazole and its derivatives. Serotonin is an important neurotransmitter identified in modulating the nociceptive response at many stages in the pain pathway. The data from animal studies suggests that serotonin (5-HT) and serotonin receptors play a role in modulating nociceptive reflexes in a complex manner. They can inhibit or activate nociceptive responses depending on the type of nociceptive stimuli, subtype of the receptor, and the dose of agonists and antagonists. Especially 5-HT_2 and 5-HT_3 receptors were reported to modulate nociceptive transmission[35, 36, 37]. In the present study, the anti - nociceptive effect of 6 – nitroindazole alone was reversed by ondensetron pretreatment (Fig- 3). This observation suggests that, tryptaminergic may be involved in the action of this compound only and not in the action of other tested compounds. Dopaminergic system has been implicated in the modulation of pain perception. An increase in dopamine turn over in specific neuronal areas and enhanced activity of descending dopaminergic pathways as a consequence of noxious stimulus has been suggested by Millan (2002)[36]. Further, intrathecal administration of dopaminergic agonist apomorphine in rats has been shown to elicit an anti-nociceptive action mediated by dopaminergic D_2 receptors[38, 39]. Clinical application of these findings has led to the use of dopaminergic agonists like apomorphine and levodopa in severe pain associated with thalamic syndrome[40] and herpes zoster[41]. A dopaminergic antagonist haloperidol has been shown to potentiate the anti-nociceptive effect of investigated indazoles (Fig- 4). Conflicting results were obtained in the present study. This observation is unique and raises the possibility whether dopaminergic antagonism could enhance the analgesic effect of indazoles. However, more detailed studies are warranted before suggesting any detailed explanation for this observation. Among several modulatory mechanisms that operate in the pain pathway, a prominent role has been suggested for the regulation of K^+_{ATP} channels. The participation of K^+_{ATP} channels in the anti-nociceptive effect of morphine at several sites in the pain pathway like peripheral, spinal and supra spinal levels have been clearly established[42]. In addition, a variety of anti-nociceptive agents like diclofenac[43], clonidine[44], $5HT_{1A}$ agonist[45] and resveratrol[46] have been reported to involve K^+_{ATP} channels in their action. In the present study, glibenclamide pretreatment significantly attenuated the anti-nociceptive effect of indazole and its derivatives clearly indicating a role for K^+_{ATP} channels in their action (Fig- 5). In conclusion, the results of the present study reveal a novel anti-nociceptive property of indazole, 5 – aminoindazole and 6 – nitroindazole against three different models of nociception in mice. Among the tested compounds; maximal antinociceptive effect was observed with 5 – aminoindazole against three nociceptive assays. Multiple mechanisms have been identified to contribute to the anti-nociceptive effect of these compounds.

ACKNOWLEDGMENT

The support extended by Sree Balaji Medical College, Hospital and Bharath University for the study is gratefully acknowledged.

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