

# Evaluation of Analgesic and Anti-inflammatory Effects of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in Mice

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## Research Article

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# Abstract

**Purpose:** The purpose of the study is to evaluate the analgesic and anti-inflammatory activity of new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione and to compare its effects with the available standard drug, diclofenac sodium in mice. **Methods:** This test compound was evaluated for its anti-nociceptive activity using hot plate and writhing models, while for antiinflammatory activity evaluation, carrageenan and histamine-induced paw edema models were used. **Results:** In hotplate test, the latency time (in seconds), at 90 minutes of the administered doses (60 and 120 mg/kg) of 5-amino-3-phenyl -1.3.5-thiadiazine-2-thione were 19.5 seconds ( $p < 0.05$ ) (Mean latency time) and 21.5 seconds ( $p < 0.01$ ) (Mean latency time) respectively. In writhing test maximum inhibitory effect was demonstrated at a dose of 120 mg/kg with an average of 1.4 ( $p < 0.001$ ) wriths (this inhibition was more significant) followed by 60 mg/kg (Mean number of wriths = 1.6) ( $p < 0.001$ ) . The anti-inflammatory effect of the compound for 60 and 120 mg/kg doses was found to be significant and was more significant for 120 mg/kg in both histamine and carrageenan-induced paw edema models. **Conclusion:** It has been concluded that 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione represents, dose-dependent antinociceptive and anti-inflammatory properties, which are statistically significant. The test compound showed a significant *in-vivo* peripheral analgesic effect in the writhing test, in comparison to the diclofenac sodium.

## Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), opiates and related drugs are widely used for the treatment of pain, inflammation fever and also arthritis (Talley et al. 2000; Palomer et al. 2002). The pharmacological effect of NSAIDs is related to the suppression of biosynthesis of prostaglandin from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs) (Smith et al. 1998; Warner et al. 1999). COX plays a vital role in the production of prostaglandins and the release of chemical pain mediators; therefore, inhibiting COX will reduce the painful response resulting from the prostaglandin cascade (Shakir et al., 2012). COX exists in two isoforms, COX-1 and COX-2, which are activated differently (Marnett and Kalgutkar 1998, 1999; Dannhardt and Kiefer 2001). COX-1 provides cytoprotection in the gastrointestinal (GI) tract whereas inducible COX-2 mediates inflammation (Prasit and Riendeau 1997; Habeeb et al. 2001; Almansa et al. 2003). As most of the NSAIDs in the market show greater selectivity for COX-1 than COX-2 (Jackson and Hawkey, 1999), long term use of NSAIDs, may lead to GI irritation, ulceration and bleeding (Allison et al. 1992). The incidence of clinically significant GI side effects due to NSAIDs is high (30%) and causes some patients to abandon NSAID therapy (Tammara et al. 1994). GI damage from NSAID mostly involved reduced tissue prostaglandin production and local irritation by the carboxylic acid moiety, common to most NSAIDs, which weakens the protective role of prostaglandins in maintaining homeostasis and GI health (Smith et al. 1998; Hawkey et al. 2000). Synthetic approaches based upon NSAIDs and other potential drugs chemical modification have been taken with the aim of improving NSAID safety profile and effectiveness issues.

In the current study, 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione (a new synthetic compound) was evaluated for its anti-nociceptive and anti-inflammatory activities. It is a 3,5-disubstituted tetrahydro-2H

1,3,5-thiadiazine-2-thione (THTT) derivative. THTT derivatives have gained attention as they are reported for several biological activities such as antifungal, antibacterial, anticancer, antileishmanial, etc. (Rodríguez et al. 2012). Also, some other compounds having thiadiazine nucleus (mercaptotriazoles condensed with thiadiazine nucleus) have significant analgesic and anti-inflammatory activities with lower GIT problems (Ochoa et al. 1999; Hussein et al. 2001; Monzote et al. 2005; Sert-Ozgun et al. 2017).

## Methods

### 2.1. Chemicals and apparatus used

The new tested compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione) was synthesized in organic chemistry Lab., ICS (Institute of chemical sciences), University of Peshawar. Diclofenac sodium was used as a standard drug. Tween 80 was used as a suspending agent while DMSO was used as a solvent. Solutions for test compound, carrageenan, and histamine were prepared in normal saline. Harvard apparatus, USA (Hot plat apparatus) was used for hot plat test while Plethresmometer was used for the measurement of histamine and carrageenan-induced paw edema. All the chemicals were of analytical grades and were used without any further purification.

### 2.2. Animals

All the experiments were performed by using male Swiss Albino mice. These experimental animals were obtained from VRI (Veterinary Research Institute), Peshawar, KPK, Pakistan. Mice weighing between 18 and 40 g were used. The experimental animals were provided with standard laboratory conditions under 25 ° C (room temperature). They were fed on an ad libitum laboratory diet and had open access to water for drinking at standard ambient temperature conditions (25°C) in 12 hrs. Dark/12 hrs. light cycle. The study in animals was sanctioned by the Ethical Review Board of the Faculty of Pharmacy, Gomal University (No. 247/QEC/GU). All experimental animals were handled in conformity with the ethical principles (substantiated in 1979) for animals to be used in a laboratory, to serve humanity, (Lyon, France).

### 2.3. Anti-nociceptive activity

#### 2.3.1. Hot plate test

Eddy's hot plate test was used with slight modifications. In this experiment male mice were used. These mice were withdrawn from food two hours before the test begins. These mice were subjected to a pretest on a hot plate with a temperature of  $55 \pm 1$  °C. To minimize inconsistency, those animals were excluded from having the time of latency greater than 15 seconds. These animals were divided into five groups (n=4). Group-I was administered with 10 ml/kg normal saline, intraperitoneally (i.p), while Group-II was treated with the reference drug "diclofenac sodium" (20mg/kg, i.p). Group-III, IV, and V were i.p inoculated with 30, 60, and 120 mg/kg of the tested compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione), respectively. Subsequent to intraperitoneal administration, time of latency (in seconds) was recorded

after 30, 60, and 90 mins, until lifting, flicking, jumping, or licking of forepaws. To avoid tissue damages, a cut off time of 30 seconds was set (Masocha et al. 2016).

### **2.3.1. Writhing test**

The antinociceptive property of the synthetic compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione) was determined by the writhing test according to Muhammad et al. 2017 with slight modification. In this experiment, male mice were used. These mice were withdrawn from food two hours before the test. The animals were arranged in five groups (n=5, per group). The Group-I i.e. control group was given an intraperitoneal injection of 10 ml/ kg NS (normal saline) and Group-II was administered with 20 mg / kg diclofenac sodium i.p. Furthermore, Group-III, IV, and V were i.p administered with 30, 60, and 120 mg/kg, of the tested compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione), respectively. After 30 minutes time interval, the animals were administered with 0.1 ml of acetic acid (1% solution), i.p. Subsequently, for the next 10 minutes number of wriths were noted, after 5 minutes of acetic acid administration.

## **2.4. Anti-inflammatory activities**

### **2.4.1. Carrageenan induce paw edema**

This experiment was performed as portrayed by Saeed et al. 2016, with some necessary modifications. In this experiment male mice were used. The animals were withdrawn from food two hours before the test begins. These were arranged into five different groups i.e. each group having four mice. Group-I received 10 ml/kg saline, while Group II received 20 mg/kg diclofenac sodium as a reference drug. Moreover, the further three groups received 30, 60, and 120 mg/kg, (i.p.) of the tested compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione), respectively. 1% suspension of carrageenan (0.1 ml) was administered into the sub planter area of the left hind paw of mice to produce inflammation, 1 hour after intraperitoneal injection of control, standard, and tested compound. The volume of the paw was measured with Plethresmometer by 0, 1, 3, and 5 hours after carrageenan administration.

### **2.4.2. Histamine induced paw edema**

This particular test was performed per Mbiantcha et al. 2010 with some necessary modifications. In this experiment male mice were used. These mice were withdrawn from food two hours before the test begins. These animals were arranged into five different groups i.e. each group having four animals. Group-I received 10 ml/kg saline, while Group II received 20 mg/kg diclofenac sodium as a reference drug. Moreover, the further three groups received 30, 60, and 120 mg/kg, i.p of the test compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione), respectively. 1% suspension of histamine (0.1 ml) was administered into the sub planter area of the left hind paw of mice to produce inflammation, 1 hour after intraperitoneal injection of control, standard, and tested compound. The volume of the paw was measured with Plethresmometer by 0, 1, 3, and 5 hrs. after histamine administration.

## **2.5. Statistical analysis**

Data analysis was performed by using ordinary one-way ANOVA as a pre-test and Dunnett's multiple comparisons test as a post-test, in which the  $p < 0.05$  value was considered significant. All the behavioral studies were analyzed through Graph Pad Prism 8.

## Results

### 3.1. Antinociceptive activity:

#### 3.1.1. Effect of a new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in Hot Plate Test

The Fig. 2. displays the results for the analgesic effect of the new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in the hotplate model. The latency time (in seconds), at 90 minutes of the administered doses (60 and 120 mg/kg) of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione were 19.5 seconds ( $p < 0.05$ ) (Mean latency time) and 21.5 seconds ( $p < 0.01$ ) (Mean latency time) respectively. At 60 minutes of treatment, the doses of 30 and 60 mg / kg were recorded to be significant (18.075 and 18.5 seconds,  $p < 0.05$ ) but 120 mg / kg (22.3 seconds,  $p < 0.01$ ) was found to be more significant. At 30 minutes readings, only 120 mg/kg was significant, while the other two doses 30 and 60 mg/kg were insignificant.

#### 3.1.2. Effect of a new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in the acetic acid-induced writhing test

The Fig. 3 shows the analgesic effect of the new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in various doses (30, 60 and 120 mg/kg). The inhibitory effect of the test compound on wriths depends on the dose. The greatest inhibitory effect was demonstrated at a dose of 120 mg/ kg with an average of 1.4 wriths ( $P < 0.0001$ ) followed by 60 mg/kg (Mean number of wriths = 1.6,  $P < 0.0001$ ). Likewise, the inhibitory effect of the test compound on wriths at 30 mg/kg was 26.8 (Mean number of wriths).

### 3.2. Anti-Inflammatory Activity

#### 3.2.1. Effect of new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in carrageenan-induced paw edema model

Anti-inflammatory effect of the new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in the paw edema model caused by carrageenan was shown in Fig. 4. Data statistical analysis showed that the anti-inflammatory effect of the test compound depends on dose and time. At 5 hours, a maximum anti-inflammatory effect of 0.125 ml ( $P < 0.001$ ) (mean paw edema) was shown at 120 mg / kg dose, followed by 60 mg / kg showing 0.13 ml ( $P < 0.001$ ) (mean paw edema). Likewise, after 3 hours, the maximum anti-inflammatory action was observed at 120 mg/kg, which was 0.14 ml (mean paw edema), followed by 60 mg / kg (0.1475 ml= mean paw edema). While 30 mg/kg dose was found to be insignificant at all times.

### 3.2.2. Effect of new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in histamine-induced paw edema model

Anti-inflammatory effect of the new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in the paw edema model caused by histamine was shown in Fig. 5. Data statistical analysis showed that the anti-inflammatory effect of the test compound depends on dose and time. At 5 hours, a maximum anti-inflammatory effect of 0.16 ml ( $P < 0.001$ ) (mean paw edema) was shown at 120 mg / kg dose, followed by 60 mg / kg showing 0.1725ml ( $P < 0.001$ ) (mean paw edema), while 30 mg/kg dose was also found to be significant. After 1 and 3 hours, some anti-inflammatory action was observed at 120 mg/kg, while the doses of 60 mg/kg and 30 mg/kg was found to be insignificant.

## Discussion

In recent practice, NSAIDs (Nonsteroidal anti-inflammatory drugs) are the most frequently prescribed drugs all over the world in the treatment of pain and inflammation. However, all NSAIDs have some sort of risk. Therefore, the development of more effective anti-nociceptive and anti-inflammatory drugs with improved safety profile is still a very attractive area of research. Tetra hydro thiadiazine thione (THTT) derived compounds have gained special attention, as they are reported for multiple biological activities. Several researchers have explored these biological activities in different studies. A series of 3,5-disubstituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione (THTT) derivatives. Some of these compounds showed antibacterial and antifungal activities (Saglam et al., 2011). Monzote et al. 2005 tested the antiparasitic effects of ten thiadiazine derivatives. These compounds showed a strong antiproliferative activity on the parasite. In other different studies, THTT derivatives were synthesized and explored for anti-fungal, anti-cancer, anti-leishmanial, and anti-protozoal properties. Some of these compounds were found active (Ochoa et al. 1999; Hussein et al. 2001). Also, there was a significant increase in analgesic as well as an anti-inflammatory activity when thiadiazine ring was fused with mercaptotriazoles, with lessor GIT effect (Sert-Ozgur et al. 2017). Due to all these studies, we synthesized a compound with thiadiazine nucleus, checked its analgesic and anti-inflammatory properties, and got our results.

The determination of abdominal contraction caused by acetic acid is a familiar model. This model is an easy, quick, and sensitive technique for the measurement of peripheral analgesic activity. The acetic acid induced ventricular contraction assay was used to assess the analgesic property of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione (test compound). The test compound suspended the abdominal writhes significantly in mice. It is a well-known fact that the response of the abdominal contraction is highly perceptive and can ascertain the analgesic properties of the test compound. The local receptors of the peritoneal cavity are believed to be partially involved in the response to abdominal contraction. The prostanoïd system appears to be responsible for the reaction mechanism to this stimulus. Several researchers have obtained the experimental results which showed elevated levels of peritoneal fluid (PGE2 and PGF2a ) and lipoxygenase products (García et al. 2004). 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione may play a significant role in prostaglandin synthesis inhibition.

In writhing test, at a higher dose (120 mg/kg) the test compound (5-amino-3-phenyl-1,3,5-thiadiazine-2-thione) showed maximum peripheral analgesic activity, in comparison to diclofenac sodium. The hotplate test is specially designed to measure the analgesic activity of centrally acting drugs like tramadol. Like writhing test, In hotplate test, At 60 minutes of treatment, the doses of 30 and 60 mg/kg were recorded to be significant (18.075 and 18.5 seconds,  $P < 0.05$ ), however 120 mg/kg (22.3 seconds,  $P < 0.001$ ) was found to be more significant. At 30 minutes readings, only 120 mg/kg was significant, while the other two doses 30 and 60 mg/kg were insignificant. We can conclude from these results that our test compound has less central analgesic activity, rather having more peripheral activity. These observations of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione, suggested that it may have peripherally (more) and centrally (less) mediated anti-nociceptive activities. This hypothesis is consistent with those of (Eddy and Leimbach 1953; Koster 1959; Williamson and Fitter 1996; Eddouks et al. 2012) who claimed that hot-plate and acetic acid induced writhing models are appropriate and useful techniques for evaluating the centrally and peripherally anti-nociceptive agents, respectively.

We also evaluated the anti-inflammatory activities of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione by using different experimental models, such as Carrageenan/histamine induced paw edema tests. Carrageenan induced mice paw edema test is a suitable one for assessing drugs anti-inflammatory effects and frequently used to evaluate the activity of a synthetic compound against edema.

In this model, data statistical analysis showed that the anti-inflammatory effect of the test compound depends on dose and time. At 5 hours, a maximum anti-inflammatory effect of 0.125 ml ( $P < 0.001$ ) (mean paw edema) was shown at 120 mg/kg dose, followed by 60 mg / kg showing 0.13 ml ( $P < 0.001$ ) (mean paw edema). Likewise, after 3 hours, the maximum anti-inflammatory action was observed at 120 mg / kg, which was 0.14 ml (mean paw edema), followed by 60 mg/kg (0.1475 ml= mean paw edema). While 30 mg/kg dose was found to be insignificant at all times.

Another study used to evaluate the anti-inflammatory activity was histamine induced paw edema test. The results showed that the anti-inflammatory effect of the test compound depends on dose and time. At 5 hours, a maximum anti-inflammatory effect of 0.16 ml ( $P < 0.001$ ) (mean paw edema) was shown at 120 mg / kg dose, followed by 60 mg / kg showing 0.1725 ml ( $P < 0.01$ ) (mean paw edema), while 30 mg/kg dose was also found to be significant. After 1 and 3 hours, some anti-inflammatory action was observed at 120 mg/kg, while the doses of 60 mg/kg and 30 mg/kg was found to be insignificant.

Some chemicals like histamines, serotonin, and other related compounds are discharged up to 3 hours (in the 1st phase) of inflammation, whereas the increase in posterior paw volume from 3 to 5 hours describes the 2<sup>nd</sup> phase of inflammation. Some second phase inflammatory mediators are responsible for this increase in paw volume. The in vivo anti-inflammatory action of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione bared that the biphasic inflammatory incidents induced by carrageenan were significantly controlled ( $p < 0.05$ ) by the test compound and therefore it can be useful as an active anti-inflammatory agent.



As for the possible mechanisms involving inflammation, it was suggested that several mediators of inflammation like kinins, histamine, complement, pro-inflammatory cytokines and PGs (prostaglandins) perform a vital role. Migration of leukocytes to harmed tissues is known to be a significant part in the process of inflammation (Carvalho et al. 1999). At least some of these mediators are believed to be inhibited by 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione (test compound). Serotonin and histamine cause the immediate response to inflammation, while prostaglandins (PGs) and kinins are responsible for a prolonged inflammatory response.

This study revealed the anti-inflammatory and analgesic activities of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione. It is obvious from our results that the test compound possesses anti-inflammatory and analgesic properties as compared to the standard drug, diclofenac sodium, supported by animal models like carrageenan and histamine-induced paw edema tests for anti-inflammatory properties, and hotplate and acetic acid-induced writhing tests for anti-nociceptive studies. The 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione was found to have a valid safety profile as the experimental animals showed no significant side effects and mortality rate was zero at a maximum dose of 120 mg/kg.

## Conclusion

It has been concluded that 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione represents, dose-dependent antinociceptive and antiinflammatory properties, which are statistically significant. The test compound showed a significant *in-vivo* peripheral analgesic effect in writhing test, even better than that of the reference drug diclofenac sodium. This study provide a pharmacological basis for the use of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione against the inflammation and algesia. Further study is required to elaborate this pharamacological activity of of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione.

## Declarations

### Ethical Approval

The study in animals was sanctioned by the Ethical Review Board of the Faculty of Pharmacy, Gomal University (No. 247/QEC/GU). All experimental animals were handled in conformity with the ethical principles (substantiated in 1979) for animals to be used in a laboratory, to serve humanity, (Lyon, France).

### Consent to Participate

NA

### Consent to Publish

All the author of this article are agreed to publish it in "Naunyn-Schmiedeberg's Archives of Pharmacology"

## Authors Contributions

JA, ZRN and HMI conceived and designed the research, JA and KUS conducted experiments TA and KAK analyzed the data, HK, A, GA and RK wrote the manuscript, MJ revised the manuscript. All authors read and approved the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

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## Competing Interests

The authors declare no competing interests.

## Availability of data and materials

Data is available as supplementary.

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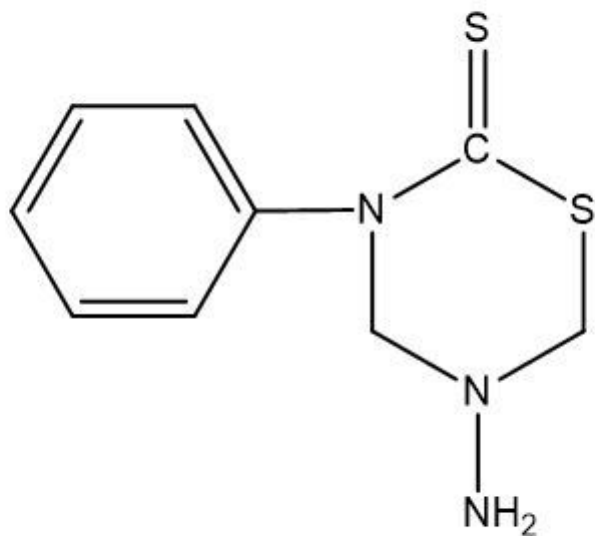
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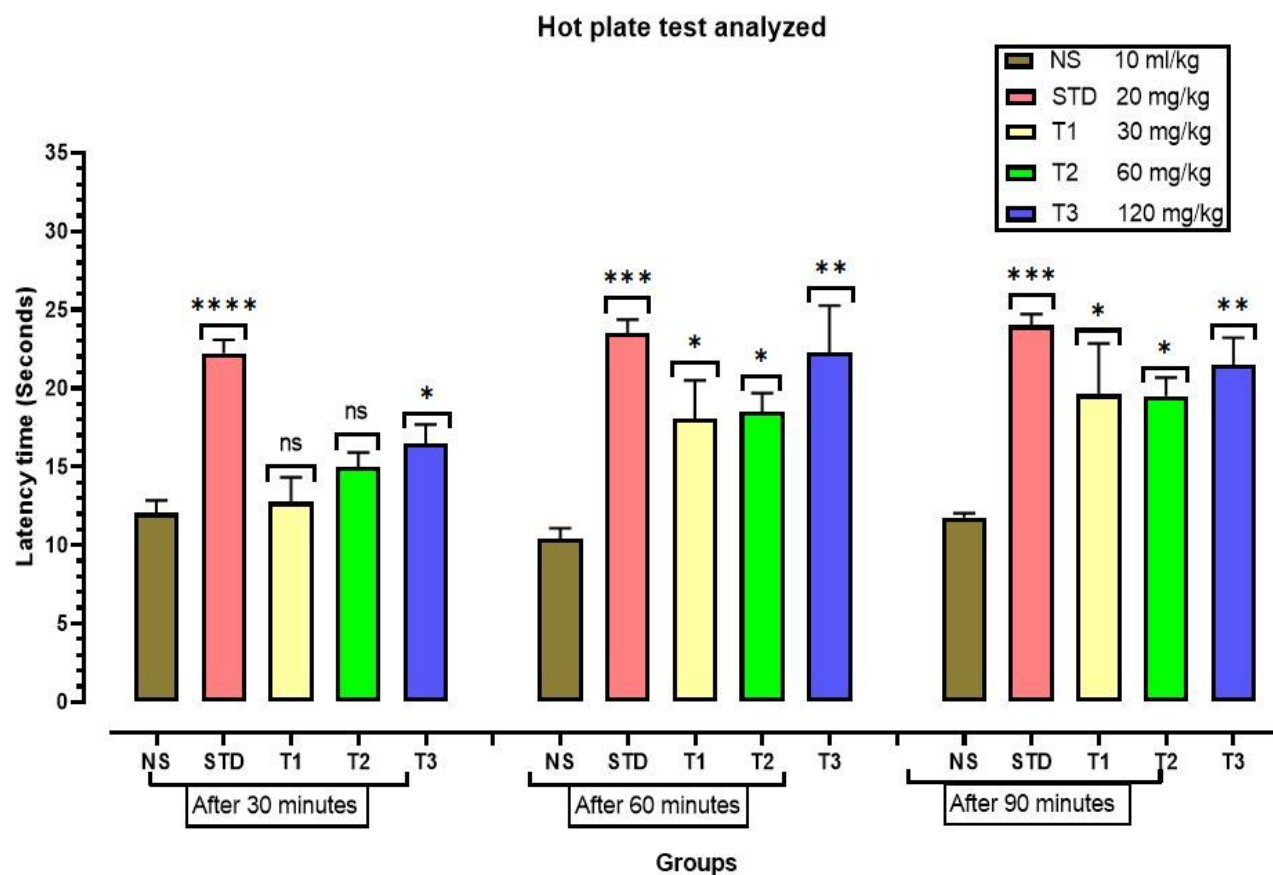
## Figures



5-amino-3-phenyl-1,3,5-thiadiazine-2-thione

### Figure 1

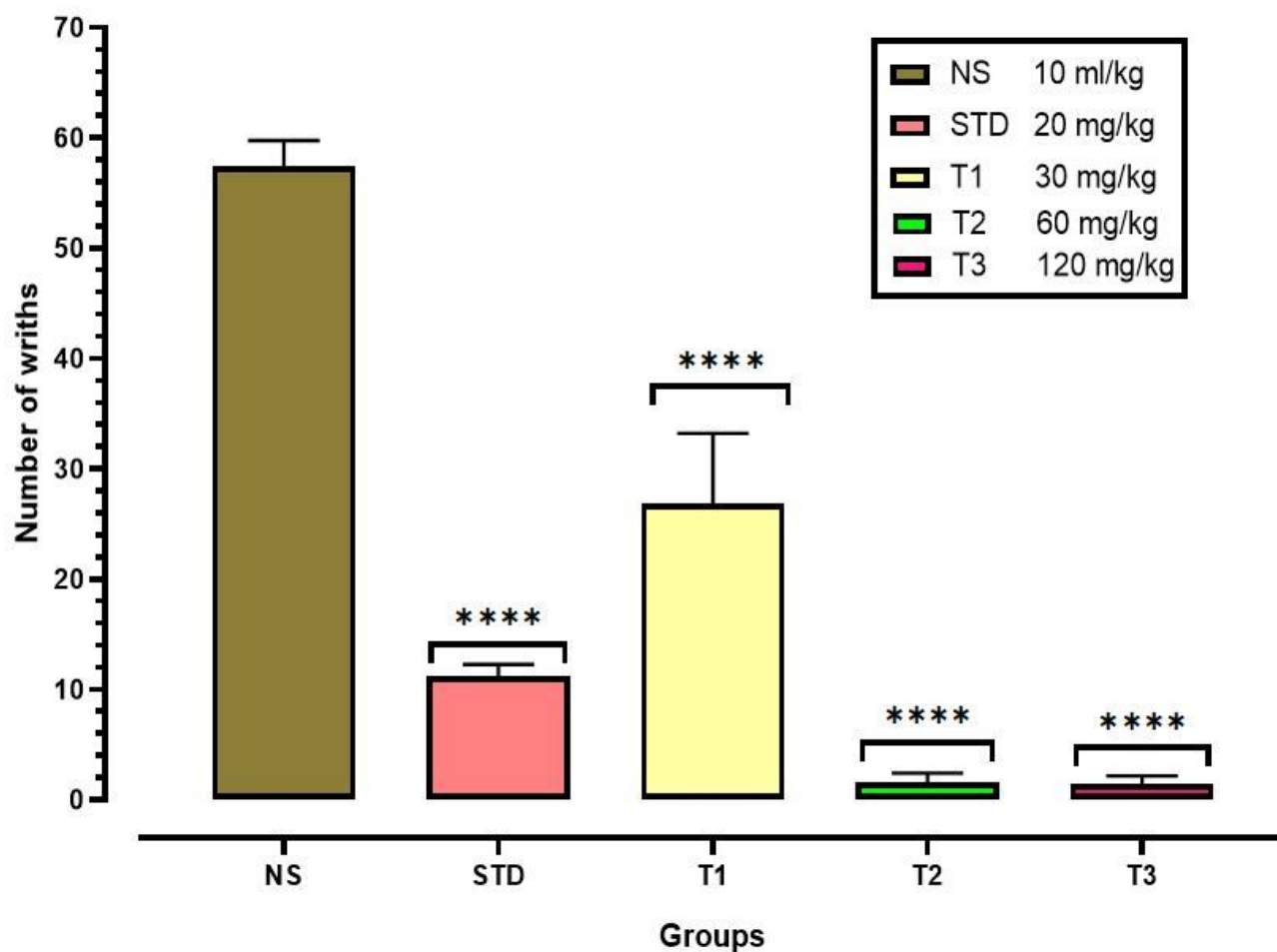
The structure of 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione



**Figure 2**

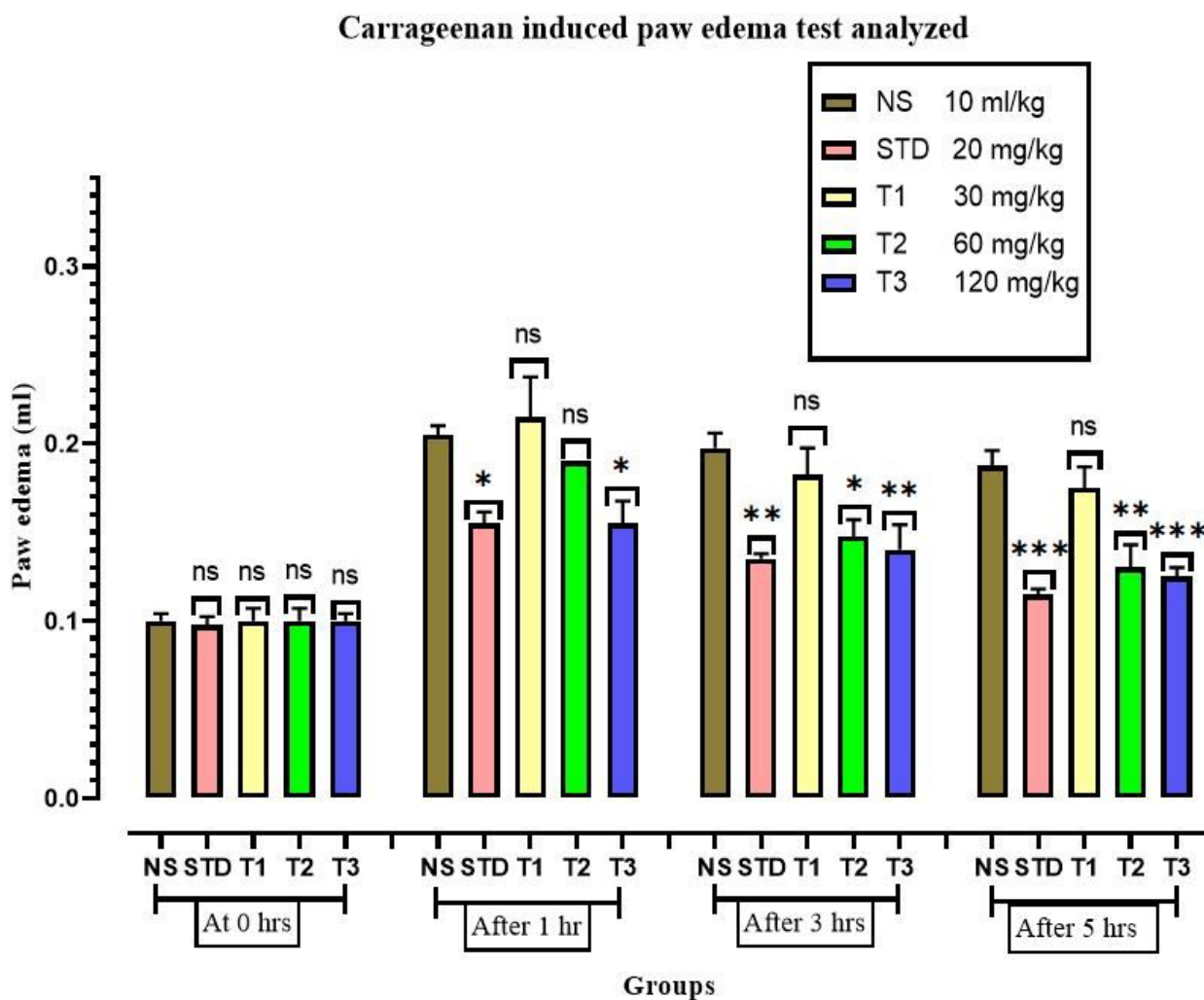
Shows the results for the analgesic effect of the new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione and diclofenac sodium in the hotplate model (n=4). Each column in the figure shows latency time (in seconds) for different treated groups. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$  and \*\*\*:  $p < 0.001$  compared to control.

### Writhing test analyzed



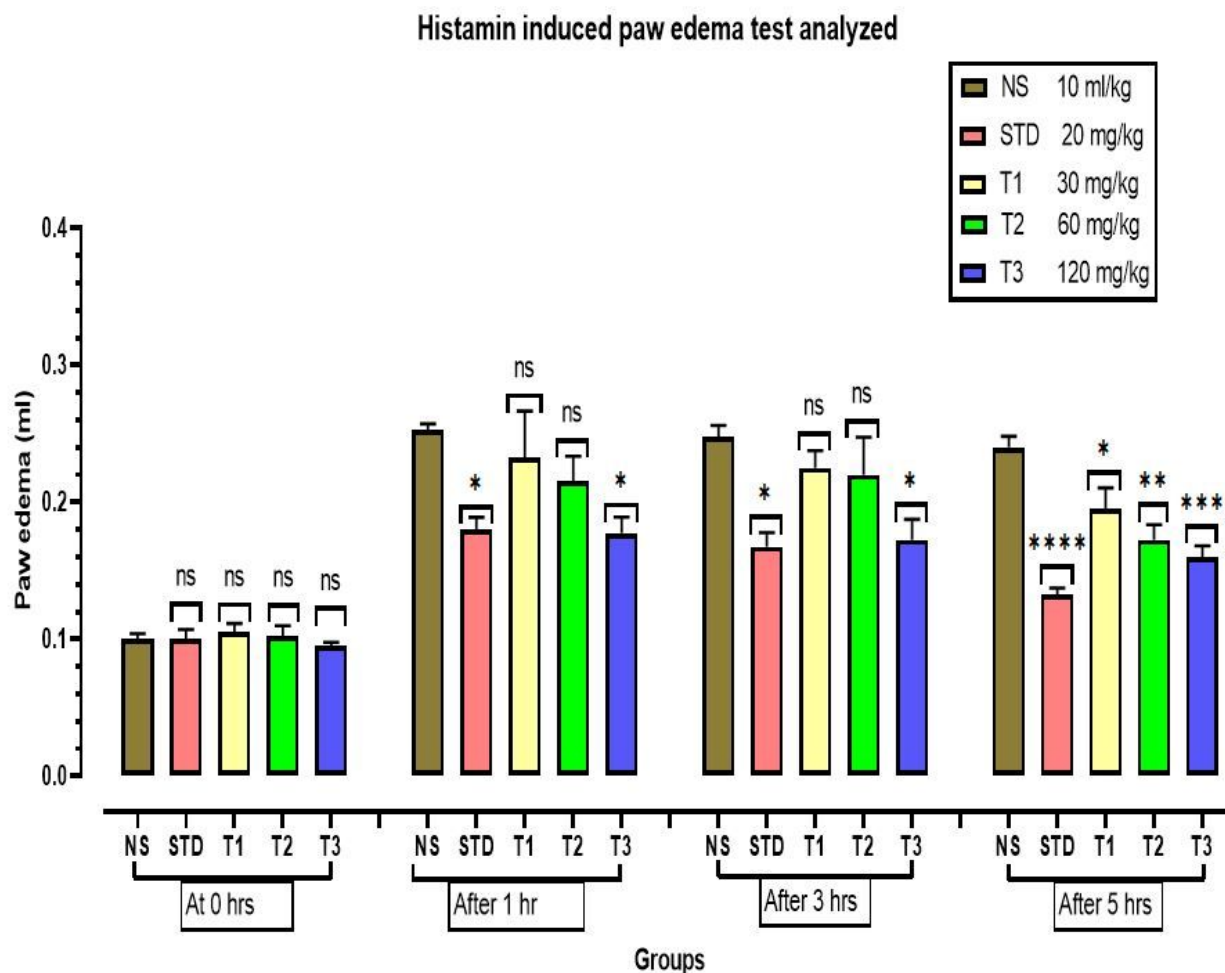
**Figure 3**

displays the effects of diclofenac sodium and new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione in acetic acid induced writhing model (n=5). Diclofenac sodium (20 mg/kg), normal saline (10 ml/kg), new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione (30, 60, and 120 mg/kg), and acetic acid (1% solution) were administered intraperitoneally. In above figure, the columns represent inhibition of wriths in animals. \*\*\*\*p < 0.001, compared with control group.



**Figure 4**

Shows the effects of diclofenac sodium (20 mg/kg) and new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione (30, 60, and 120 mg/kg) on the development of paw edema induced by carrageenan in mice (n=4). The test compound (60, 120 mg/kg) significantly inhibited the paw edema in mice, in a dose and time dependent manner at different time intervals. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$  and \*\*\*:  $p < 0.001$  compared to control.



**Figure 5**

Shows the effects of diclofenac sodium (20 mg/kg) and new synthetic compound: 5-amino-3-phenyl-1,3,5-thiadiazine-2-thione (30, 60, and 120 mg/kg) on the development of paw edema induced by histamin in mice. The test compound (60, 120 mg/kg) significantly inhibited the paw edema in mice, in a dose and time depended manner at different time intervals. \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$  and \*\*\*\*:  $p < 0.0001$  compared to control.