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PHARMACOLOGICAL EVALUATION OF BREWER'S YEAST-INDUCED ANTIPYRETIC AND ANALGESIC ACTIVITY OF *ALLIUM SCHOENOPRASUM* ON WISTAR RATS

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ABASTRACT

Pyrexia (heat) and pain are two of the most common clinical symptoms of infection, inflammation, and damage, and they often coexist to indicate underlying pathogenic processes The aim of this study was to investigate the analgesic and antipyretic properties of the ethanol extract from leaves of the plant *Allium schoenoprasum* in Wistar albino rats. The antipyretic activity of leaves of the plant *Allium schoenoprasum* was studied in Brewer's yeast-induced pyrexia in rats. The analgesic activity of leaves of the plant *Allium schoenoprasum* was studied using the tail-flick method. In analgesic activity models, ethanol extract significantly ($p < 0.001$) reduces the painful stimulus. It also possesses antipyretic activity; ethanol extract significantly ($p < 0.01$) reduces fever at higher doses within 3 hours on a Brewer's yeast-induced pyrexia model in rats. The findings of this research indicate that the ethanolic extracts of *Allium schoenoprasum* leaves exhibited antipyretic properties that were significant between the doses of 200 mg/kg and 400 mg/kg of body weight. Furthermore, it is possible that the primary mechanism(s) of action of these extracts is the inhibition of the synthesis and/or release of inflammatory mediators. Ethanolic extracts of *Allium schoenoprasum* leaves also showed analgesic activity at high doses.

KEYWORDS: Allium schoenoprasum, brewer's yeast, antipyretics, analgesic aspirin, temperature.

INTRODUCTION

Pyrexia (Heat): Elevated body temperature makes the environment less conducive for pathogens and allows lymphocytes, such as T and NK cells, to migrate and fight infections more efficiently. Pain serves as a "warning signal" to safeguard a weaker body from further harm, directing the individual to conserve energy and relax.Pyrexia (heat) and

pain are two of the most common clinical symptoms of infection, inflammation, and damage, and they often coexist to indicate underlying pathogenic processes. They are essential elements of "sickness behavior" and acute phase reactions.

Fever is widespread at all ages. It affects over 35% of hospitalized patients and up to 70% of critically ill patients in intensive care units (ICUs). Pyrexia of Unknown Origin (PUO) in adults is frequently diagnosed as infectious (e.g., tuberculosis), neoplastic (e.g., lymphoma), or autoimmune (e.g., adult-onset Still's disease).[1] The management of fever generally prioritizes improving the patient's comfort rather than entirely eliminating the fever, as the fever itself can play a role in augmenting the body's immune response. Treatment approaches often encompass a combination of pharmacological and non-pharmacological therapies. Among the first-line pharmacological treatments are antipyretic medications like acetaminophen (paracetamol) or non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen. These medications function by lowering the set point of the hypothalamus, which leads to a decrease in body temperature and provides relief from associated pain.[2] In addition to medication, non-pharmacological methods for managing fever are equally important. Adequate hydration is crucial because fever can lead to fluid loss through sweating. Drinking fluids such as water, broths, and electrolyte solutions can help combat this fluid loss. Additionally, patients are advised to rest sufficiently to allow the immune system to function optimally. When it comes to physical cooling methods, lukewarm baths or applying cool, damp cloths to areas such as the forehead and armpits can offer significant relief. However, it is vital to exercise caution and avoid ice baths or immersing oneself in cold water. These approaches can trigger shivering, which may paradoxically raise the core body temperature rather than lower it.[3]

Plant secondary metabolites are naturally occurring organic compounds that are not directly involved in plant growth or reproduction but play essential roles in defense and interaction with the environment. These compounds are of great pharmacological importance due to their wide range of therapeutic activities, including antipyretic (fever-reducing) and analgesic (pain-relieving) properties. Major classes of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, glycosides, and saponins have been extensively studied for these effects. Fever is typically caused by the release of pyrogens that stimulate the production of prostaglandins in the hypothalamus, leading to an increase in body temperature. Many plant-derived compounds, particularly flavonoids and phenolics, exert antipyretic activity by inhibiting cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis and helping to normalize body temperature. Additionally, their antioxidant properties help reduce oxidative stress and inflammation, which are often associated with fever.[4]

Analgesic activity of plant secondary metabolites involves the reduction of pain through both central and peripheral mechanisms. Alkaloids are well known for their action on the central nervous system, where they may interact with opioid receptors to block pain perception. On the other hand, flavonoids, tannins, and terpenoids act mainly at the peripheral level by inhibiting inflammatory mediators such as prostaglandins, histamine, and bradykinin, which are responsible for causing pain and swelling. Terpenoids, commonly found in essential oils, also exhibit anti-inflammatory and mild sedative effects, contributing to pain relief. Tannins help in reducing inflammation by precipitating proteins and forming a protective layer over tissues, thereby decreasing irritation and pain. Glycosides and saponins further enhance these effects through their anti-inflammatory and immune-modulating properties.[5]

Several medicinal plants are rich in these bioactive compounds and have been traditionally used for treating fever and pain. For instance, Azadirachta indica contains flavonoids and tannins that contribute to its antipyretic and analgesic effects. Ocimum sanctum is another important medicinal plant known for its eugenol content, which has strong anti-

inflammatory and pain-relieving properties. Similarly, Zingiber officinale contains gingerols that help reduce inflammation and pain, while Curcuma longa contains curcumin, a potent compound with both antipyretic and analgesic activities. These plants have been widely used in traditional medicine systems and are increasingly being validated by modern scientific research. One of the key advantages of plant secondary metabolites is their relatively lower risk of side effects compared to synthetic drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), which may cause gastric irritation or kidney issues with prolonged use. Plant-based compounds often work through multiple mechanisms and may exhibit synergistic effects, enhancing their overall therapeutic efficacy. However, proper dosage, standardization, and clinical validation are necessary to ensure their safety and effectiveness. plant secondary metabolites are valuable natural sources of antipyretic and analgesic agents. Their ability to modulate inflammatory pathways, inhibit prostaglandin synthesis, and interact with the nervous system makes them effective in reducing fever and pain. With increasing interest in natural and herbal medicine, these compounds continue to play a significant role in drug discovery and the development of safer therapeutic alternatives.[6]

MATERIAL AND METHODS

Plant Collection and Extract Preparation - Fresh *Allium shoenoprasam leaves* were collected from [local nursery], identified and authenticated by a botanist- Dr. Sandeep K. Verma (Professor and Head of Dept. in Botany) SAGE University Indore (M.P.) [Voucher No. J/Bot/SLF-037]

Extraction

The leaves of plant *Allium schoenoprasum* are collected and shade dried. Coarse powder is made from these dried leaves and subjected to extraction in increasing polarities. Extract is prepared by using suitable solvents like ethanol.

Each crude extract obtained after removing the solvent will be subjected to preliminary phytochemical screening and ethanolic extract is subjected for anti- pyretic and analgesic activity. The ethanolic extracts of leaves are prepared by the process of Soxhlet apparatus. Soxhlet is the process of extraction of a powder drug with a solvent. The collected leaves were washed 2-3 times with tap water to remove adhering dust and allowed to dry in shade. The dried material was crushed to coarse powder with mechanical grinder. It was then passed through the 40 No. mesh sieve. The powder was stored in airtight container. A weighed quantity (150 gm) of the powder was subjected to continuous hot extraction in Soxhlet apparatus with ethanol as a solvent and extracted till the solvent became colourless. Ethanoic extract of *Allium schoenoprasum* leaves (EASL) was evaporated under reduced pressure using desiccator at a low temperature of 40-60°C until the extract turned syrupy and then this syrupy extract was transferred to an evaporating dish for drying on a water bath The extraction was carried out for a period of 72 extract obtained was dried in vacuum to remove excess solvent and were weighed for the determination of % yield[7]

Preclinical studies

The animals maintained under standard environmental conditions had free access to standard diet and water ad libitum. Rats were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 hour light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.[8]

Approval of Animal Ethical Committee

The study was conducted after obtaining from committee for the purpose of control and supervision on animals (CPCSEA) and institutional animal ethics committee (IAEC), proposal number IAEC/SVCP/2022/02. For this experiment, 30 albino rats of either sex weighing between 150 and 200 grams were obtained from the Animal House of SVCP Indore. They were split up into six groups (n = 5). Seven days prior to the trial starting, they were acclimated to the laboratory environment and given unrestricted access to water and a typical dry pellet diet. The Institutional Animal Ethics Committee (IAEC) authorized the experimental procedure for the use of animals in this study. Before every experiment, the animals were given unrestricted access to water and fasted for the whole night.

Table 1. Study Design

S. No	Group	Group	Dose, drugs, and Schedule
1	Group I	Control	control group was given 1ml of water
2	Group II	Negative control	Negative control was treated with 20 ml/kg brewer yeast alone
3	Group III	Standard	standard reference was treated with brewer yeast and with 100mg/kg of Aspirin
4	Group IV	EASL Low dose 200 mg	was treated with brewer yeast and 200mg/kg of ***Allium schoenoprasum***
5	Group V	EASL high dose 400 mg	was treated with brewer yeast and 200mg/kg of ***Allium schoenoprasum***

Evaluation anti pyrexia caused by brewer's yeast

The extract was then dissolved in 0.8% Tween 80 to make a solution of concentration 100mg/ml of *Allium schoenoprasum* leaves (EASL). The extract was then given orally to the groups of animals in the appropriate dosages of 200 mg/kg-.400 mg/kg. Aspirin was dissolved in distilled water to make a solution of 75mg/ml and was given orally to the appropriate groups of animals in the correct dosage (100mg/kg). Albino rats were divided into 5 groups of five rats in each group. Fever was induced by injecting 20ml/Kg (subcutaneous) of 20% suspension of brewer's yeast in normal saline below the nape of the neck (Somezeet et al., 2009). The temperature was measured after 18hours using rectal thermometer. The temperatures were taken at 0, 1, 2, 3 and 4 hours after various substances were administered.[9]

Evaluation of Analgesic Studies by Tail Flick method

A metal artery clip was applied to the root of animal's tail (1cm from the body) to induce pain. A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 15 sec. were discarded. Analgesic activity was evaluated 0, 30, 60, 90, and 120 min. after oral administration of the extracts and controls. An artery clip is placed at the root of tail and a positive analgesic response was indicated if animal attempt to dislodge the clip by biting the clip or tail within 5 sec. in any of the consecutive trials. The reaction time between application of the clip and response is noted by a stopwatch. The mean value was evaluated.[10]

RESULT AND DISCUSSION

Phytochemical Investigation

The Ethanolic extract of *Allium schoenoprasum* leaves (EASL)was subjected to preliminary Phytochemical screening for the presence of different Phytoconstituents such as alkaloids, saponins, glycosides, tannins, flavonoids, carbohydrates etc. The presence of Carbohydrate was confirmed by Fehling's test, Benedict test, Seliwanoff's test, Tolen's test. The presence of protein & amino acids was confirmed by Million's test & Wagner's test. The presence of steroids by Salkowski reaction, Liberman Burchard reactions & Tannins by 5% FeCl3 solution whiles the Glycoside by

Legal's test, Keller Kilani test and Flavonoids by lead acetate test. As, the ethanolic extract show the presence of most of these compounds, these extracts were selected for the study.

Table 2: Phytochemical Analysis leaves of plant Allium schoenoprasum.

Phytochemicals	Observation
• Carbohydrates	-
• Proteins	+
• Phytosterols	+
• Glycosides	+
• Tannins	-
• Phenols	+
• Flavonoids	+
• Alkaloids	+
• Volatile oil	-
• Vitamines	+

Evaluation anti pyrexia caused by brewer's yeast

Table 3: Evaluation anti pyrexia extract of Allium schoenoprasum leaves (EASL) rat caused by brewer's yeast.

Group	Treatment & dose	Initial temp.	Rectal temperature after 18hours of yeast injection (°C)				
			0 hour	1 hour	2 hours	3 hours	4 hours
GI	Normal Saline 1ml/kg	36.61±0.36	36.57 ±. 29	36.59±0.25*	36.55 ±0.26*	36.57 ±0.30*	36.51±0.31*
GII	Brewer's yeast 20ml/kg	36.70±0.33	38.01 ±0.30	38.26±0.25	38.91 ±0.27	37.90 ±0.18	37.85±0.19
GIII	Aspirin100 mg/kg	36.39±0.23	36.73 ±0.51	36.15±0.41	36.84 ±0.29	36.61 ±0.24*	36.51±0.23*
GIV	*EASL dose 200 mg*	36.64±0.37	37.95 ±0.47	37.88±0.67	37.11 ±0.32	36.93 ±0.52	37.81±0.34
GV	*EASL dose 400 mg*	35.91±0.71	37.39 ±0.48	37.50±0.27	36.75 ±0.31*[a]	36.11 ±0.31*	36.09±0.25*[a]

Values are Mean± S.E.M. (n=5) Significance vs. Negative control group: *P<0.001. a= when compare to group 2

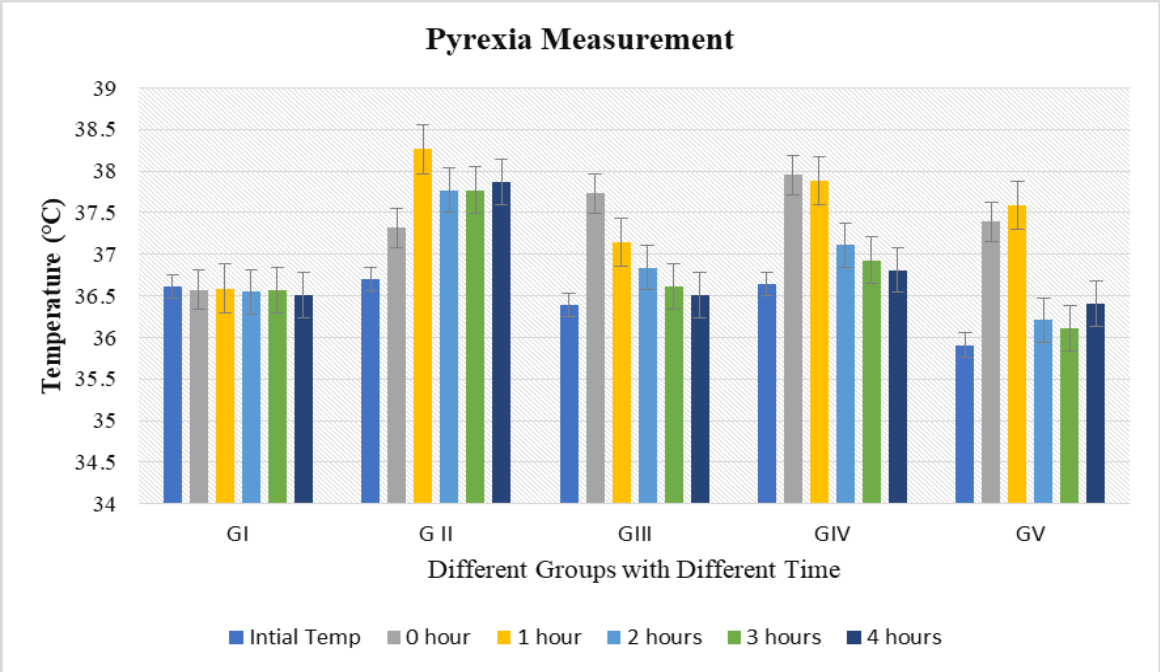

Figure 1: Pyrexia Measurement.

Evaluation analgesic activity by Tail Flick Test

Table 5: Effect of EASL Extract on tail flick test.

Treatment (mg/kg)	Latency Period ± SEM				
	0 min	30 min	60 min	90 min	120 min
Control	3.12 ± 0.15	3.14 ± 0.24	3.22 ± 0.22	3.58 ± 0.22	3.59 ± 0.18
EASL dose 200 mg	4.13 ± 0.23	4.78 ± 1.49	4.00 ± 1.87	3.62 ± 1.78	2.58 ± 0.88
EASL dose 400 mg	3.26 ± 0.12	5.94± 0.44 *	5.96 ± 0.91 *	6.44 ± 0.58*	6.98 ± 1.17*
Diclofenac 10 mg/kg	3.45 ± 0.17	7.19 ± 0.65 *	9.30 ± 1.45 **	10.68 ± 1.47 **	9.78** ± 0.64
Values are represented as means ± SEM (n=4 in each group) Data expressed by using one way ANOVA followed by Dunnett's Test. Value are show Significance vs. Negative control group: *P<0.005. and **P<0.001 when compare to group 2					

The table shows how the ethanolic extract of *Allium schoenoprasum* leaves affected the tail flick test. This study mentions the extract at a dose of 200,400 mg/kg, as well as standard diclofenac at 10 mg/kg. The study found that diclofenac sodium 10 mg/kg had a significant effect on the control group. Ethanolic extract from *Allium schoenoprasum* leaves. There is no significant difference between the control and the EASL 200 mg doses. There is a significant difference between the EASL doses of 400 mg.

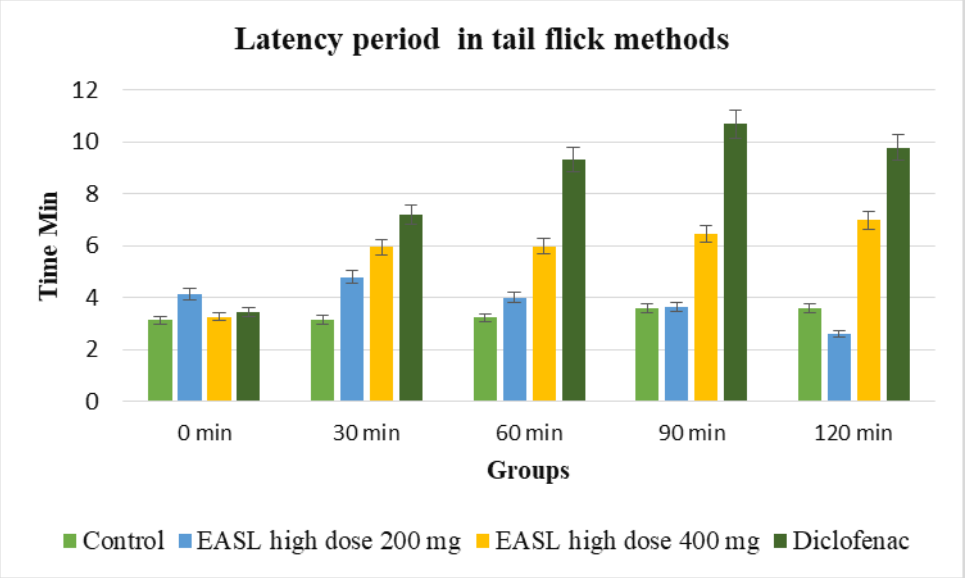

Figure 2: Pyrexia Measurement.

DISCUSSION

The results of the antipyretic effect of the different doses of the test compound (200mg/kg,400mg/kg), standard(aspirin,100mg/kg), negative control and control are depicted in Table 7.2. Aspirin as well as ethanolic extract at doses of 200mg/kg, 400mg/kg started showing effective antipyretic activity after 1h of post dosing; while at dose of EASL 200mg/kg the effect was not as significant as that of aspirin, when compared with the control. Comparing all other groups with the negative control, there was significant reduction in the temperature. Antipyretic activity was observed up to 4h after aspirin and test extract administration. EASL high dose 400 mg show significate difference when compare to both negative control and normal control group.

Fever is known to be caused by several endogenous pyrogens such as interleukin-1β, interleukin-6, interleukin-8, tumor necrosis factor-α, macrophage protein-1 and prostaglandins. Prostaglandin synthesis may be activated by tumor

necrosis factor-α and phospholipase A2. Brewer's yeast induces both TNF-α and prostaglandin synthesis (Rititid et al., 2007). It is currently accepted that prostaglandin E2 (PGE2) is the final fever mediator in the brain, specifically in the preoptic area of the anterior hypothalamus (Li et al., 2008). Antipyretics such as aspirin (acetylsalicylic acid) and other non-steroidal anti-inflammatory drugs (NSAIDs) reduce fever by suppressing peripheral production of interleukin-1β, while consecutively lowering the thermoregulatory set point by blocking central cyclo oxygenase formation of prostaglandin E2. Thus, it can be inferred that inhibits the synthesis of prostaglandins. It has been established that there are two pathways leading to the transcription and induction of cyclo-oxygenase (COX)-2. Both pathways are activated by cytokines e.g. IL-1α, IL-6 and tumor necrosis factor (TNF) which trigger central mechanisms that act via the transcription factors such nuclear factor (NF)kB and signal transducer and activator of transcription (STAT-3) (Inoue et al., 2008). However, additional studies will be needed to determine if the antipyretic mechanism was due to inhibition of TNF-β synthesis or prostaglandin synthesis or both. Phytochemical analysis from previous studies has shown that Allium schoenoprasum contained flavonoids Flavonoids belong to the polyphenol family and are found in most plant material. The most important dietary sources are fruits, tea and soybean. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti inflammatory and anti-viral A variety of flavonoids have also been found to inhibit prostaglandin synthase (COX-2) production and transcription Plants such as Palisota hirsuta K. Schum. (Commelinaceae) and Jasminum Trichotomum contain flavonoids and have been found to possess antipyretic properties Hence the presence of flavonoids might account for the antipyretic effect that was observed in ***Allium schoenoprasum.***

In this present study, found Flavonoids, phenols and alkaloids. etc. The Flavonoids and phenolic compound have Analgesic (pain-relieving) activity is caused by a number of phytochemicals, mostly from classes like alkaloids, terpenoids, and flavonoids. They interact with nervous system receptors, inhibit inflammatory enzymes, and alter pain pathways. By lowering inflammation, blocking enzymes like COX-2, or interacting with pain receptors, these plant-based compounds frequently provide relief comparable to that of conventional medications with fewer adverse effects. Thus, the ability of the leaf extract to prolong the latency period for pain indicates activity via central pain pathways, probably through the modulation of endogenous substances that target inflammation and pain.

CONCLUSION

Pyrexia or fever is a disease caused as a result of secondary impact of other diseased states due to the resetting of the hypothalamic set-point. From scientific discovery, antipyretic drugs such as aspirin, NSAIDs, opioids have been developed for use and of which mostly produces side effects including gastrointestinal bleeding, renal, hepatic effect, etc. Therefore, many herbal plants have been found to be having antipyretic effects. *Allium schoenoprasum is* a shrub commonly found in whole world and has been used to treat bacterial fevers locally. This study investigates the antipyretic activity of the ethanoic extract of *Allium schoenoprasum* brewer's yeast induced fever in experimental rats.

25 albino rats weighing 150g-200g were used. They were divided in to 5 groups of five rats each. Group one serve as control (n=5) and was given 1ml of normal saline, group two (n=5) was treated with brewer yeast alone, group three(n=5) was given 100mg/kg of asipirin, while groups four, five and five were treated with 400mg/kg,200mg/kg (n=5) of *Allium schoenoprasum* respectively. A suspension of brewer's yeast was injected subcutaneously to induce fever in all the experimental animals. After 18hrs, the rectal temperature was taken and the animals were administered *Allium schoenoprasum* (200mg/kg.400mg/kg,) and aspirin (standard group, 100mg/kg) orally. The body temperature of

the rats was measured rectally over a period of 4hours. *Allium schoenoprasum* (200mg/kg, and 400mg/kg) significantly reduced yeast induced pyrexia, when compared with the group two (20ml/kg, brewer's yeast). Antipyretic activity was observed up to 4h after aspirin and test extract administration. EASL high dose 400 mg show significate difference when compare to both negative control and normal control group. Thus, this experiment shows that the antipyretic effect *Allium schoenoprasum* is dose dependent and the effect is as a result of the flavonoid component of the extract.

These data therefore suggest that ethanoic extract of *Allium schoenoprasum* leaves possesses significant antipyretic activity and it mechanism could be by inhibition of release inflammatory mediators. In conclusion, the findings of this research indicate that the ethanolic extracts *Allium schoenoprasum* leaves exhibited antipyretic properties that were significant between the doses of 200 mg/kg and 400 mg/kg of body weight. Furthermore, it is possible that the primary mechanism(s) of action of these extracts is the inhibition of the synthesis and/or release of inflammatory mediators ethanolic extracts *Allium schoenoprasum* leaves also shown analgesic activity at high dose.

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