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Evaluation of Ethanolic Root Extract of *Allium Cepa*. L For Analgesic and Anti-Inflammatory Activities in Animal Models

Krupavaram.B, Kaashmeera Nair.K, Hanish Singh. JC, Anandarajagopal K, Jaasminerjiit Kaur, Shopana Paramasivam.

School of Pharmacy, KPJ Healthcare University College, Perserian Seriemas, Kota Seriemas, Nilai 71800, Negeri Sembilan, Malaysia.

ABSTRACT

Objectives: This present study was envisaged to identify the effect of analgesic and anti-inflammatory activity forethanolic root extract of *Allium cepa* L.

Methodology: The study was carried out using Sprague- Dawley rats (250- 300g) and Albino mice (25- 30g). Diclofenac sodium and Tramadol are the standard drugs, was prepared by dissolving in distilled water to make the concentration of 10mg/kg and 20mg/kg for anti-inflammatory and analgesic activity respectively. The effect of ethanolic extract of *A.cepa* L root was investigated for analgesic activity using tail immersion method. The anti-inflammatory activity of ethanolic root extract of *A.cepa* was studied using carrageenan-induced paw edema to the rats.

Results: The anti-inflammatory activity was evaluated using carrageenan method in Sprague- Dawley rats. The anti-inflammatory activity was found to be dose dependent in carrageenan induced paw edema model. The ethanolic extract has shown highly significant ($p < 0.001$) percentage of inhibition of paw edema, 57.43% and 60.56% at 5th hour at the low dose and high dose, respectively. Whereas analgesic activity was studied using tail immersion method in mice. The ethanolic root extract was shown highly significant ($p < 0.001$) analgesic activity with standard drug and high dose but, low dose showed less significant ($p < 0.05$) analgesic activity.

Conclusions: The findings of the present study concluded that *A.cepa* L roots have potential to treat pain and inflammation and as a good source, novel natural analgesic and anti-inflammatory agents. The ethanolic extract of *A.cepa* L root showed highly significant analgesic and anti-inflammatory activity in mice and rats respectively.

Key words:

Allium cepa L, Analgesic, Anti-inflammatory, Tail immersion, and Carrageenan.

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*Corresponding Author

Name: Krupavaram. B

Email: kruparajs@gmail.com

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INTRODUCTION

Analgesics commonly known as painkillers and used to relieve the pain without make anaesthesia. Analgesia is a state incited on administering analgesics and can be characterized as diminishing from pain. Analgesia is an unpleasant sensory and produced by the excitation of specific receptors. Pain relieving infers from Greek (without) and algos (hurting). Pain relieving drug act in several ways on the peripheral and CNS. Pain can be categorized as a chronic or acute. Acute pain is a symptom of pain, but chronic pain is a disease (Sinatra, Jahr et al. 2010).

The word inflammation comes from the Latin word "inflammar" which means state of being inflamed or warmth associated with redness and swelling. Inflammation is a cognitive process by which the body's white blood cells and part they produce assist us from infection with foreign organisms, such as bacteria and viruses. There are two types of acute and chronic inflammation. Acute inflammation starts in seconds after minutes of tissue injury, damage may be solely physical, or it may involve activating the immune response. Chronic inflammation refers to a prolonged response to inflammation that involves progressive multiple types of cells present in inflammation (Dorsch, Schneider et al. 1990). *Allium cepa* L used as herbal medicine or a vegetable to eat either raw or for cooking purpose and it has great illness importance and consumed for its supposed nutritional and health benefits. *A.cepa* L was commonly known as an onion. The *A.cepa* L family of Liliaceae is an expansive

and assorted one containing more than 500 species. It has four consumable wild plants it could have advanced from all of which develop in the focal Asian district. *A.cepa* L is one of the exceptionally nutritious vegetables with ethno-restorative value in Asia countries, which is normally known as Shallot (China), Vengayam (India), Cepolla (Italy), Hom khao (Thailand) and bawang (Malaysia) (Ravi.K et al.,2016). Phytochemical studies of *A.Cepa* L extract have presence of flavonoids, flavonols, quercetin, fructose, querceti-3-glucoside, isorhamnetin-4-glucoside, xylose and cysteine sulfoxides, cycloalliin, selenium, thiosulfinates, essential oil, sulfur, seleno compounds and more. *A.Cepa* L has an uncommon combination of three groups chemical constituent that exacerbates accepted such as fructans, flavonoids, and organosulfur mixes. The first active ingredient in onion is quercetin, which is one of the properties of flavonoids exhibits antioxidant properties that good (Dorsch, Schneider et al. 1990). As the *A.cepa* L have anti-oxidant potential, it may reduce the inflammation. In the present study, *A.cepa* L has been selected which is a very popular medicinal agent in the ethnic drug. But scientific data regarding the use of *A.cepa* L for analgesic and anti-inflammatory activity is not available for whole roots. Hence this topic has been chosen to evaluate whether does *A.cepa* L has the ability to empower anal gesic and anti-inflammatory activity.

1. METHODOLOGY

1.1 PLANT MATERIAL

Red onion (*Allium cepa* L) roots were obtained from the supermarket, Seremban, Negeri Sembilan in the month of January 2017. The plant material was identified and authenticated by Dr. Mohd Firdaus Ismail, Biodiversity Unit, Institute of Bioscience, University Putra Malaysia, Serdang, Selangor Darul Ehsan, Malaysia. A voucher specimen was deposited at herbarium, UPM, Serdang. (RefNo; UPM/IBS/UB/H64/18).

1.2 PREPARATION OF EXTRACTS

After collection *Allium cepa* L roots were washed thoroughly with tap water then rinse with sterile water. The roots were dried initially under shade for 4 weeks at room temperature and powdered. The dried root was subjected to size reduction to a coarse product by using a dry grinder and passed through size no: 30 and stored in a tightly closed container. The fresh coarse powder was used for ethanolic extraction by cold maceration method. About 250g of coarse powder was extracted by cold maceration process by using 500ml of ethanol. The sample was soaked for 7 days with vigorously shaken thrice daily at the proper interval. The extraction was filtered using Whatman no 1 filter paper and dried using rotary evaporator (below 50%), then placed in the tightly closed container and in the dry place.

1.3 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical screening was conducted for the extract of *Allium cepa* L roots using suitable methodologies to identify the presence of plant secondary metabolites such as alkaloids, carbohydrates, and glycosides, protein and amino acids, oils and fats, saponins, terpenoids, flavonoids, sterols, phenolic compounds, tannins as well as gums and mucilages.

1.4 ANIMALS

Sprague-Dawley rats weighing 250- 300g and Albino miceweighing 25- 30g were procured from vivarium of KPJ Healthcare University College, Kota Seriemas, Nilai, Negeri Sembilan Darul Khusus. and housed in polypropylene rat & mice cages. All rats and mice were fed diet food and water. The animal was maintained under temperature ($22^{\circ}\text{C} \pm 3$), humidity ($60 \pm 5\%$) and a 12 hour light and dark cycle. The study was authorized by Institutional Research and Ethics Committee of KPJ Healthcare University College. (Ref No: KPJUC/RMC/BPS/EC/2018/153).

1.5 ANTI-INFLAMMATORY

The rats were divided into four groups (n=6) and fasted for 12 hours prior to the experiment. The anti-inflammatory activity of various extracts of *A.cepa* L roots was assessed by carrageenan-induced hind paw edema method with slight modification using digital plethysmometer. Prior to any treatment, each rat was weighed properly and the initial paw volume was noted for each rat. Rats in group I received injected carrageenan (1% w/v) and served as negative control group. Rats in group II received the standard drug, Diclofenac sodium (10mg/kg) orally 30 min prior to administration carrageenan. Group III and IV was received the *Allium cepa* L roots ethanol respective extract (200mg/kg and 400mg/kg) and served as low dose and high dose. Carrageenan (1% w/v), administered in subplantar region of right hind paw each rat were

administered orally to the animals. The low dose, high dose, and standard drug were administered orally 30 min before the carrageenan-injected to each rat. The paw volume was measured at 0, 30, 60, 120, 180 and 240 min after the administration of the extract and standard drug. The anti-inflammatory activity was assessed by observing the paw edema volume after 30 minutes of carrageenan administration. (Anar Patel et al.,2010)

1.6 ANALGESIC

Animal were divided into four groups (n=6) and each mice fasting for 12 hours' prior before the experiment. The analgesic activity of varied extracts of *Allium cepa* L roots was assessed by the tail immersion method. Prior to any treatment, each mouse was weighed properly and the tail immersion reading was noted for each mice. The group I given orally normal saline 1 % serve as a control. Groups II were given standard drugs Tramadol respectively (20 mg/kg) was administered orally, half an hour before the screening. The ethanol extract of *Allium cepa* L roots was given orally 200mg/kg as low dose to group III and group IV received ethanol extract of *Allium cepa* L roots 400mg/kg as high dose, one hour before start the tail immersion method. Placing the mice tail up to 1-2 cm into the beaker of hot water which maintained at ($55^{\circ}\text{C} \pm 0.5$). The mice withdraw its tail from the hot water known as the response of pain was taken as the response time; the cut off time of tail immersion was set at 15 seconds and recorded the response after 0, 30, 60, 90 and 120 min after administration of extract and standard drug. (S.K Sharma et al.,2004)

1.7 STATISTICAL ANALYSIS

All the outcome were communicated as mean \pm SEM. Statistical significance was analysed by ANOVA followed by Dunnett "T" test, the statistical analysis which was conducted with SPSS software. The huge contrast between the gatherings will be resolved to utilize an examination tests with the estimation of $p < 0.05$ which considered as statistically significant.

2. RESULTS

3.1 Preliminary phytochemical analysis

Based on preliminary phytochemical screening conducted, both analgesic and anti-inflammatory activity with ethanolic extract of *Allium cepa* L roots showed the presence of alkaloids, carbohydrates, and glycosides, phenolic compound, and tannins, flavonoids, volatile oil and terpenoids.

3.2 Result of Anti-inflammatory activity

Anti-inflammatory activity of *Allium cepa* L roots extract was carried out by using the carrageenan-induced rat paw edema method. The anti-inflammatory activity of ethanolic extract of *Allium cepa* L roots on Sprague Dawley rats with a range of 200 - 250 g/kg b.wt. The result were compared with standard conventional anti-inflammatory drug, Diclofenac sodium at the concentration of 10 mg/kg b.wt were administered orally for 30 minutes before the carrageenan induction and followed the same procedure for *Allium cepa* L ethanolic root extract for low dose and high dose at the level of 200mg/kg and 400mg/kg respectively. In the present study, the ethanolic extracts exhibited a reduction in paw volume induced by carrageenan. The edema percentage of the inhibitory activity was figured by the following equation:

$$\text{Percentage of the inhibitory} = \left(\frac{T_o - T_t}{T_o} \right) \times 100$$

Where:

Tt = Represents the increase in paw volume in the test group

To = Represents the increase in paw volume in the control group

After the oral administration of the extract for the experiment animals, the anti-inflammatory activity was observed from first hour onwards. After the injection of carrageenan in the right hind paw of each animal, there was a gradual increase in paw volume was observed in the right hind paw of each animal up to 30 min. The efficacy of ethanol extract of *Allium cepa L* root extracts in decreasing the inflammation level of carrageenan-induced paw edema in Sprague- Dawley rats were demonstrated in Table 3.2.1.

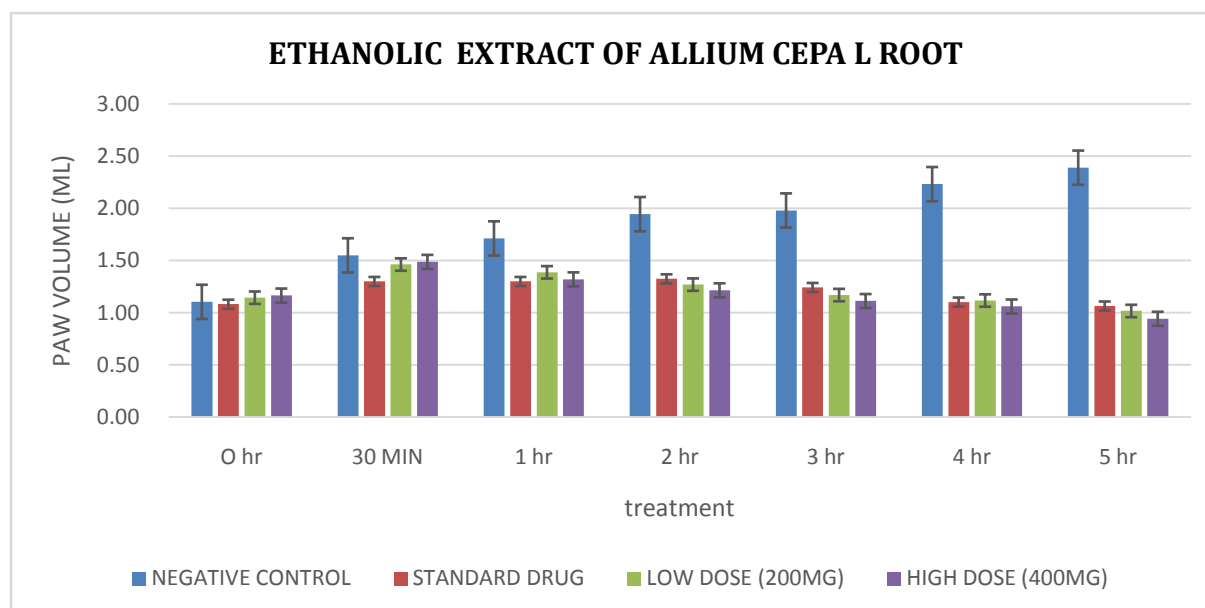
The effect of ethanolic root extract of *Allium cepa L* on the carrageenan-induced rat paw edema at different hours (30 min, 1, 2, 3, 4 and 5 hours) of study was compared with negative control for the evaluation of anti-inflammatory activity based on account of the percent inhibition of paw edema volume as showed in Table 1. The group I with carrageenan-induced treatment show

TREATMENT (MG/KG)	PAW VOLUME (ML) (MEAN ± SEM)							Inhibition (%) (5 hours)
	Initial Paw Volume (0 Hour)	30 Min	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	
Negative Control	1.10 ± 0.04	1.55 ± 0.004	1.71 ± 0.23	1.94 ± 0.15	1.98 ± 0.04	2.23 ± 0.08	2.39 ± 0.07	-
Standard Drug	1.08 ± 0.02	1.30 ± 0.05 **	1.30 ± 0.07 *	1.32 ± 0.03 *	1.24 ± 0.04 **	1.10 ± 0.04 ***	1.06 ± 0.04 ***	55.44 %
Low Dose 200mg/kg	1.14 ± 0.02	1.46 ± 0.07	1.38 ± 0.04 *	1.27 ± 0.01 **	1.17 ± 0.02 **	1.11 ± 0.03 ***	1.01 ± 0.0 ***	57.43 %
High Dose 400mg/kg	1.16 ± 0.02	1.48 ± 0.02 *	1.32 ± 0.02 *	1.21 ± 0.05 **	1.11 ± 0.05 ***	1.06 ± 0.02 ***	0.94 ± 0.08 ***	60.56 %
TREATMENT (MG/KG)	PAW VOLUME (ML) (MEAN ± SEM)							Inhibition (%) (5 hours)
	Initial Paw Volume (0 Hour)	30 Min	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	
Negative Control	1.10 ± 0.04	1.55 ± 0.004	1.71 ± 0.23	1.94 ± 0.15	1.98 ± 0.04	2.23 ± 0.08	2.39 ± 0.07	-
Standard Drug	1.08 ± 0.02	1.30 ± 0.05 **	1.30 ± 0.07 *	1.32 ± 0.03 *	1.24 ± 0.04 **	1.10 ± 0.04 ***	1.06 ± 0.04 ***	55.44 %
Low Dose 200mg/kg	1.14 ± 0.02	1.46 ± 0.07	1.38 ± 0.04 *	1.27 ± 0.01 **	1.17 ± 0.02 **	1.11 ± 0.03 ***	1.01 ± 0.0 ***	57.43 %
High Dose 400mg/kg	1.16 ± 0.02	1.48 ± 0.02 *	1.32 ± 0.02 *	1.21 ± 0.05 **	1.11 ± 0.05 ***	1.06 ± 0.02 ***	0.94 ± 0.08 ***	60.56 %

Table 1: Anti-inflammatory activity of extract of *Allium cepa L* root on carrageenan-induced paw edema method

Values are expressed as mean \pm SEM,; (n=6),*** p<0.001- Highly significant,** p<0.01- Significant and * p<0.05- Less significant as compared to control and *Allium cepa L* ethanolic root extract

Figure 1. Anti-inflammatory activity of extract of *Allium cepa L* root on carrageenan-induced paw edema method



Result of Analgesic activity

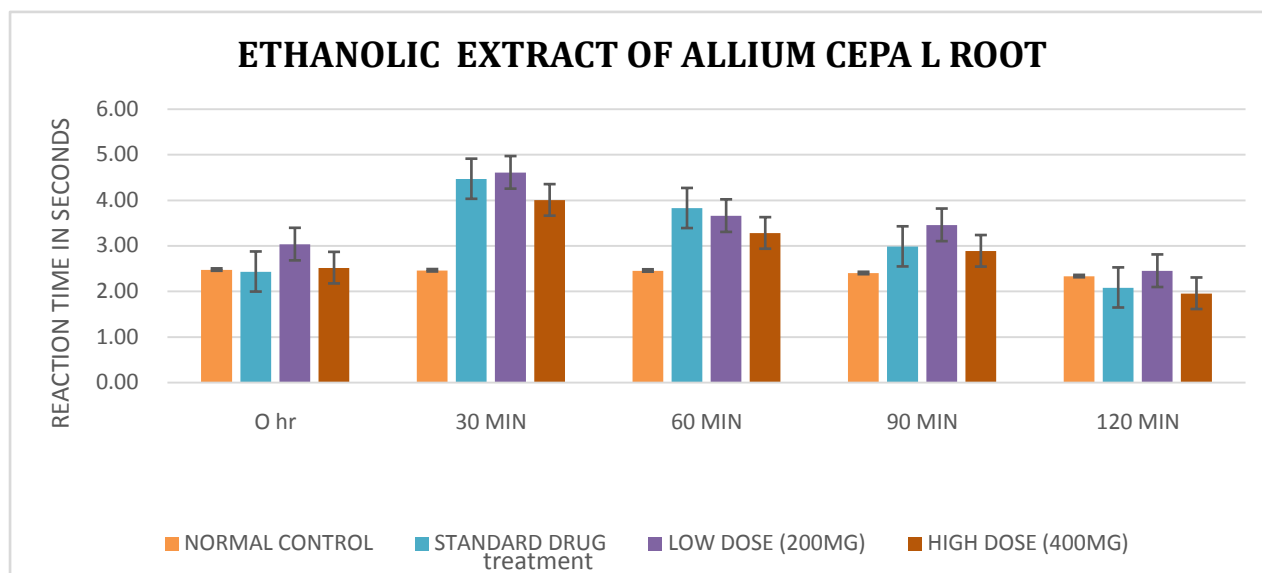
Analgesic activity of *Allium cepa L* roots extract was carried out by using tail immersion method. The analgesic activity of ethanolic extract of *Allium cepa L* roots on Albino mice was studied at the concentration 25 g/kg b.wt. The result was compared with the standard conventional analgesic drug, Tramadol at the concentration of 20 mg/kg b.wt was administered orally for half an hour before the screening and followed the same procedure for *Allium cepa L* ethanolic root extract for low dose and high dose at the level of 200mg/kg and 400mg/kg respectively. The low dose, as well as high dose, significantly showed analgesic activity, which prevented the increase the responses of withdraw its tail whereas high dose shows good significance compared to the low dose which is almost comparable to the standard drug.

The effect of ethanolic root extract of *Allium cepa L* was studied in Albino mice by observing the analgesic activity that response to withdraw its tail. The study as showed in Table 2 stated that the extract exhibited its action at dose of 200mg/kg and 400mg/kg from 30 minutes of administration of *Allium cepa L* root extract. The effect of ethanolic root extract of *Allium cepa L* on the tail immersion at different hours (30, 60, 90 and 120 minutes) of the study was compared to that of control for the evaluation of analgesic activity based on account of the reaction time of tail withdraw as showed in Table 2.

The standard drug tramadol 20mg/kg shows the pain action and elevated level of pain was observed from this statistical analysis. Then, group III and group IV showed a good significant analgesic activity of *Allium cepa L* ethanolic root extract which started to increase the reaction time of withdraw tail from 60 minutes to end of 120 minutes. The high dose is more significant compared to the low dose which is almost comparable to the standard drug.

Table 2: Analgesic activity of extract of *Allium cepa L* root on tail immersion method

TREATMENT (MG/KG)	Initial time (0 Hour)	REACTION TIME IN SECONDS (MEAN \pm SEM)			
		30 MIN	60 MIN	90 MIN	120 MIN
Normal control	2.47 \pm 0.52	2.46 \pm 0.52	2.45 \pm 0.50	2.45 \pm 0.50	2.33 \pm 0.49
Standard Drug	2.43 \pm 0.45	3.48 \pm 0.48	4.82 \pm 0.54 *	5.72 \pm 1.29 **	6.49 \pm 0.44 ***
Low Dose 200mg/kg	3.03 \pm 0.63	3.45 \pm 0.60	4.61 \pm 1.22	4.66 \pm 0.31*	4.73 \pm 0.34*
High Dose 400mg/kg	2.47 \pm 0.52	4.88 \pm 0.20*	5.28 \pm 0.07 ***	6.00 \pm 0.11**	7.28 \pm 0.25***

Figure 5.3.1: Analgesic activity of extract of *Allium cepa L* root on tail immersion method

DISCUSSION

In the main, preliminary phytochemical analysis is an essential steps need to be conducted to check the presence of important phytoconstituents in medicinal plants that are responsible for certain activities that imposed by the medicinal plant (manjulika et al.,2014) including in the root of *Allium cepa L* and subsequently drive to future discovery and development of plant based drugs. Besides, an earlier study conducted, the preliminary phytochemical analysis was done toward the *Allium cepa L* that being extracted revealed presence of alkaloids, carbohydrate, glycosides, phenolic compound, tannins, flavonoids, volatile oil, terpenoids and saponins (Mohamed Eltaweel.,2013) where all the phytoconstituents are present except saponins. In this present phytochemical screening, ethanolic extract additionally showing the presence of flavonoids, tannins and phenolic compound. The result revealed that ethanol extract of *Allium cepa L* roots has a positive anti-inflammatory and analgesic activity due to the presence of flavonoids, phenolic compound, and tannins. The present study investigated the biological effects of its extract, primarily the inflammatory and painful process. The present data clearly showed that extract of *Allium cepa L* roots have anti-inflammatory activity and analgesic activity, a good significant response was observed on inhibiting the edema formation after carrageenan sub-plantar injection and increase the reaction time for withdrawal of tail in tail immersion method. Anti-inflammatory activity of natural product in animal studies was assessed by inducing the inflammatory mediators using external stimuli such as dextran, histamine, carrageenan, and formalin. Initially inflammation was observed during the first and second hour is attributed to release histamine and serotonin, whereas the second phase is related to the release of prostaglandin and slow reacting substance which peaks at 3rd hour (Anar Patel et al., 2010). The extract which showed the highest anti-inflammatory activity with highly significant statistic values ($p < 0.001$) for carrageenan-induced edema inhibition after the treatment. The present study establishes the anti-inflammatory activity of extract of *A.cepa L*. Negative group rats (treated with carrageenan 1%) did not show any significant value for carrageenan-induced edema. The ethanol extract of *A.cepa* at low dose (200 mg/ kg) and high dose (400 mg/ kg) showed highly significant action in reducing the paw volume. The ethanolic extract of *A.cepa L*

both low dose and high dose showed high percentage of inhibition at 5th hour and the high percentage of inhibition than standard drug. The low of paw volume, the faster of inflammation reaction. Analgesics are the drugs, which selectively relieve pain by acting in the CNS and peripheral pain mediators without changing consciousness. The analgesic may be narcotic or non narcotic. Both peripheral and central pain models are included to make the test more evident for the analgesic property of the plants. Nociception is the mechanism; whereby noxious peripheral stimuli are transmitted to the central nervous system and nociception fibre terminate the superficial layers of the dorsal horn, forming synaptic connections with transmission neurons running to the thalamus (Mi Ezeja et. al., 2011). Thus, the extract which showed very good analgesic activity with highly significant statistic values ($p < 0.001$) for tail immersion test after the treatment. The present study establishes the analgesic activity of extracts of *A.cepa L*. The ethanol extract of *A.cepa L* at high dose (400 mg/ kg) showed high significant in the reaction of tail withdraw, whereas the ethanolic extract of *A.cepa L* at a low dose (200 mg/ kg) showed less significant analgesic activity. According to the study by (Ajaykumar et al., 2012) on anti-inflammation activity and analgesic activity reveals the presence of flavonoids and its potent antioxidant are well reported. By acting on both proliferative and exudative phase of inflammation, flavonoids exhibit their anti-inflammatory activity and analgesic activity. Certain flavonoids play the enzyme activities arachidonic acid metabolizing enzymes such as phospholipase A, cyclooxygenase, lipoxygenase, and nitric acid synthase. Inhibition of these enzymes by flavonoids reduces the production of crucial inflammatory mediators such as arachidonic acid, prostaglandins, leukotriene, and nitric oxide. This finding assists the present of flavonoids in roots of *A.cepa L* and it might be more responsible for the anti-inflammatory and analgesic potential of *A.cepa L* roots Furthermore, the ethanolic extract showed more effective in *A.cepa L* which might be due to the presence of flavonoids in ethanol extract.

CONCLUSION

The findings of the present study concluded that *A.cepa L* roots have the potential to treat pain and inflammation and as a good source novel natural analgesic and anti-inflammatory agents.

The current study leads to the search for novel analgesic and anti-inflammatory activity from natural products. The significant analgesic and anti-inflammatory activity were observed in ethanolic extract of *A. cepa* L roots. The ethanolic extract of *A. cepa* L root showed highly significant analgesic and anti-inflammatory activity in mice and rat. It might be due to the presence of flavonoids. Extensive scientific research on *A. cepa* L is needed to deal with the isolation of bioactive constituents, responsible for analgesic and anti-inflammatory activity and assessing the possible mechanism of action.

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CONFLICT OF INTEREST

No conflict of interest

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