Anti-convulsion, Analgesic and anti-inflammatory effect of *Lagenaria siceraria (linn.)* and carbamezapine

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Abstract

The aim of present study is to evaluate the anti-convulsion, analgesic and anti-inflammatory effect of *Lagenaria siceraria* and carbamezapine. *Lagenaria siceraria* as a medicinal plant, its fresh juice have not been examined for its activity till date. Due to the side effects of carbamezapine, have been used to evaluate its anti-convulsion, analgesic and anti-inflammatory effect in comparion through oral route. Anti-convulsion activity were performed by inducing convulsion with pilocarpine 2%, analgesic and inflammatory activities were studied by means of acetic acid induced writhing test and formalin test respectively. Treated animals received oral dosage of *L. siceraria* (400mg/kg) and carbamezapine (6mg/Kg). Anti convulsant results showed that *L. siceraria* did not protect convulsion but it produced delayed tremors. Analgesic activity showed that CBZ and *L. siceraria* produced significant reduction in pain in comparison with the standard drug (aspirin). *L. siceraria* produced 87.5% and 83.3% reduction of pain in writhing test. It also exhibited significant analgesic activity in formalin induced licking and biting test. CBZ produced 87.5 % analgesic effect in peripheral phase and 100% in neurogenic phase in writhing test, while in licking and biting test CBZ also reduced pain (58.6% and 74.1% respectively in first and second phase). In Combination group pain reduction response was increased which reflected the significance of both drugs as an analgesic agent. CBZ and *Lagenaria siceraria* both reduced inflammation in formalin induced hind paw edema. The reduction of inflammation with *L. siceraria* decrease upto 44.8% and 64.5% in both phase. CBZ decrease 58.6% phase 1 and 74% respectively with phase 1 and 2. Whereas, the combination group markedly reduced inflammation with synergistic effect.

Conclusion: *Lagenaria siceraria* fresh juice delayed the symptoms of convulsion and produce significant analgesic and anti-inflammatory effect through oral route, in combination with carbamezapine

Keywords

Lagenaria siceraria, anti-convulsion, analgesic, anti-inflammatory, carbamezapine

Introduction

In spite of the vast number of drugs, there is no ideal antiepileptic agent with properties like broad spectrum activity, minimal side effects, good oral bioavailability and low cost. The present day carbamezapine exert adverse effects, which includes liver abnormalities such as cholestatic, hepatocellular jaundice, Myocardial infarction, edema, glycosuria, elevated BUN, renal failure, aplastic anemia and agranulocytosis (Monaco *et al.*, 1976, Spickett *et al.*, 1996). Evidence of medicinal plants used in various traditional systems has been documented because of their safety and lack of toxic effects and are gaining popularity in most of the developing countries

Lagenaria siceraria (Mol.) Standl**.** commonly known as Bottle gourd (Urdu: Lauki;) has been widely used as vegetable in Pakistan. It is an annual herbaceous climbing plant with a long history of traditional medicinal uses in many countries, especially in tropical and subtropical regions. It is one of the excellent fruits gifted by the nature, having composition of all essential constituents required for good health (Rahman, 2003, Kubde et al., 2010).

Carbamezapine is used in inflammation induced epilepsy. *L. siceraria* used in combination with carbamezapine, can be very effective in reducing inflammation and producing analgesic effect.

The pharmacological activities of *Lagenaria siceraria* is well known for diabetes, colitis, piles, ulcer, jaundice, hypertension, cardiac failure, rheumatism and insomnia (Prajapati *et al.*, 2010). Analgesic and anti-inflammatory effect is compared with carbamezapine. Literature review showed that almost all of the pharmacological effects were evaluated on different solvent extracts of *L siceraria*. Till date there is no evaluation on fresh juice. Interesting results were observed with *L. siceraria* fresh juice without peel (400mg/kg approximately equal to 3.5ml fresh juice).

Material and Method

Anti convulsion

This activity investigated the impact of CBZ and *L. sisceraria* on the epileptic seizures. 400 mg/Kg of pilocarpine was injected intraperitoneally in order to induce seizures in rats. The focus was on observing the latent period for the development of convulsions and mortality rate. Activity was observed for 30 minutes and the time was noted for each response.

Analgesic activity

Writhing test (Acetic acid induced)

Writhes mean violent coiling or twisting of animal body due to pain. Writhes were induced by administering 0.6% acetic acid solution i.e., 10ml/kg intraperitonealy (Koster, 1959).

Five groups of mice (n = 5) were used. Acetic acid solution was injected intraperitoneally after thirty (30) minutes of the oral administration of tested substances. Immediately after the

administration of acetic acid, number of writhes were counted and recorded for 30 minutes. Reduced number of writhes were considered as the evidence for the presence of analgesic effect. It was calculated in terms of percent inhibition of writhing by using the following formula

$$\%\ Inhibition\ of\ writhing = \left(\frac{[Wc - Wt]}{Wc}\right) \times 100 \qquad (Eq.\ 1)$$

Where,
W_c – Count of writhing in control group
W_t – Count of writhing in treated group

Licking and biting (formalin induced)

Swiss albino mice were divided into five groups which themselves were comprised of 5 mice each. Treatments were given according to the following scheme

G 1: Normal saline G 2: Aspirin as standard drug with formalin G 3: CBZ with formalin G 4: *L. siceraria* (3.5 ml ~ 400mg/kg) with formalin G 5: CBZ + *L. siceraria* with formalin.

20 microliter of 2% w/v formalin was injected in the right hind paw. Test drugs were orally administered before 30 minutes of formalin injection. After injecting the formalin, excessive licking and biting of right hand paw was observed (Hunskaar *et al.*, 1985).

Anti inflammatory activity

The anti-inflammatory effect of CBZ, *L. siceraria* and their combination was calculated by hind paw edema induced by formalin (Apu *et al.*, 2013, Zulfiker *et al.*, 2010).

Hind paw (edema) induced by formalin
Swiss albino mice were divided into five groups (n=5), and the reading of different groups were observed for 3 hours.

G 1: Normal saline (control group), **G 2**: Aspirin with formalin,**G 3**: CBZ with formalin, **G 4**: *L. siceraria* with formalin, **G 5**: CBZ + *L. siceraria* with formalin.

Volume of the paw was measured after every 30 minutes for 3 hours by using manual vernier caliper. The following formula was used to determine the percent inhibition of edema:

$$\%\ Inhibition\ of\ edema = \left(\frac{[Vc - Vt]}{Vc}\right) \times 100 \qquad (Eq.\ 2)$$

Where,

V_c – Paw volume in control group

V_t – Paw volume in treated group

Statistical Analysis

ANOVA for windows was applied for data analysis and statistically analyzed by one-way analysis of variance. Data were presented as mean ± Standard error of the mean. $P<0.05$ was taken as level of significance. $P<0.001$ was taken to be the level of highly significance.

Results

Anti-convulsion Activity

The development of these effects was recorded with respect to the stages in which they were developed. Stage 1 includes time and frequency of vertigo, stage 2 includes body twitching count, stage 3 where seizures occurs and stage 4 determines whether the treated animal survived or died. In the control group, the first seizure occurred at 17.41 ± 0.24 min, whereas CBZ treated groups rat did not exhibit seizure, *L. siceraria* treated rats induced its first seizure at 21.44 ± 0.70 min. Combination of CBZ + *L. sisceraria* did not produce seizures as well. During first hour after injecting the pilocarpine, no death of the treated rats was observed. However control rat died after 4 hour whereas CBZ treated group survived, *L. siceraria's* fresh juice treated rat died after 6 hours and combination of CBZ + *L. siceraria* treated rat also survived.

Following the administration of 200mg/Kg of 2% w/v pilocarpine, a series of behavioural alterations have developed over 1-2 hours that includes hypoactivity, tremor, scratching, head bobbing, and myoclonic movements of the limbs which then progressed to the recurrent myoclonic convulsions with rearing, salivation with froth, body jerks with falling. In the control group the first seizure occurred at 17.41 ± 0.24 min, salivation with froth occurred at 11:17 min and 31 times body jerks. Whereas CBZ with pilocarpine treated rats did not exhibit seizures and the only symptoms that appeared was head bobbing, tremors, salivation and paw licking. In *L. siceraria* treated rats first seizure was appeared at 21.44 ± 0.70min, salivation with froth occurred at 12:08 minute and body jerk was 5 times. However, combination of CBZ + *L. sisceraria* did not produce any seizures during the test period, and it also delayed the pre symptoms of seizure whereas piolo recertion, wet dog shake, rearing, salivation with froth and body jerk were not appeared.

Table 1: Anticonvulsant activity in pilocarpine induced rats

	Stage 1 Vertigo	**Stage 2 Body twitch count**	**Stage 3 Seziures count**	**Stage 4 Death/ Survived**
Control (Pilocarpine induced)	1 min 29 sec	23 ± 1.92	4 ± 1.05	Dead
CBZ (6mg/kg)	3min 39 sec	6 ± 0.05	0 ± 0.01	Survived
LSFJ (400mg/kg)	2 min 28 sec	13 ± 1.87	5 ± 1.01	Dead
CBZ + LSFJ (6mg/kg + 400mg/kg)	3 min 28 sec	4 ± 1.42	0 ± 0.01	survived

CBZ – Carbamazepine, LSFJ – *Lagenaria siceraria's* fresh juice

Analgesic Activity

Analgesic activity of CBZ, *L. siceraria* and their combination was determined by acetic acid as well as formalin method. It is observed that all treated groups were better than the aspirin group in reducing no. of writhes, significantly (Table - 2). Combination of CBZ and *L. siceraria* were found more effective in reducing no. of lick/bites as compared to groups treated with either CBZ or *L. siceraria* alone. These results suggest better analgesic activity when CBZ is given along with *L. siceraria* for long term.

Table - 2 : Effect of Carbamezapine, *Lagenaria siceraria* fresh juice and their combination on analgesic activity (acetic acid method).

	Mean no. of writhes (0-15 min)	**Mean no. of writhes (15-30 min)**
Control 0.6% acetic acid (I.P)	16 ± 2.16	6 ± 1.74
Aspirin (300mg/Kg)	4 ± 0.92	11 ± 0.96
% inhibition	75%	83.3%
***p* value**	$p<0.0001$	$p<0.0001$
LSFJ (400mg/kg)	2 ± 1.77	1 ± 4.18
% inhibition	87.5%	83.3%
***p* value**	$p< 0.0001$	$p<0.0001$
CBZ (6mg/kg)	2 ± 0.58	0 ± 00
% inhibition	87.5%	100%
***p* value**	$p<0.0012$	$p<0.1000$
CBZ + LSFJ (6mg/kg + 400mg/kg)	1 ± 1.24	1 ± 1.78
% inhibition	93%	83.3%
***p* value**	$p<0.0001$	$p<0.0001$
LSFJ – *Lagenaria siceraria*, CBZ – Carbamazepine, *I.P* - Intraperitoneal. $p<0.05$ is significant ; $p<0.001$ highly significant Values are expressed in Mean ±SEM; n=5		

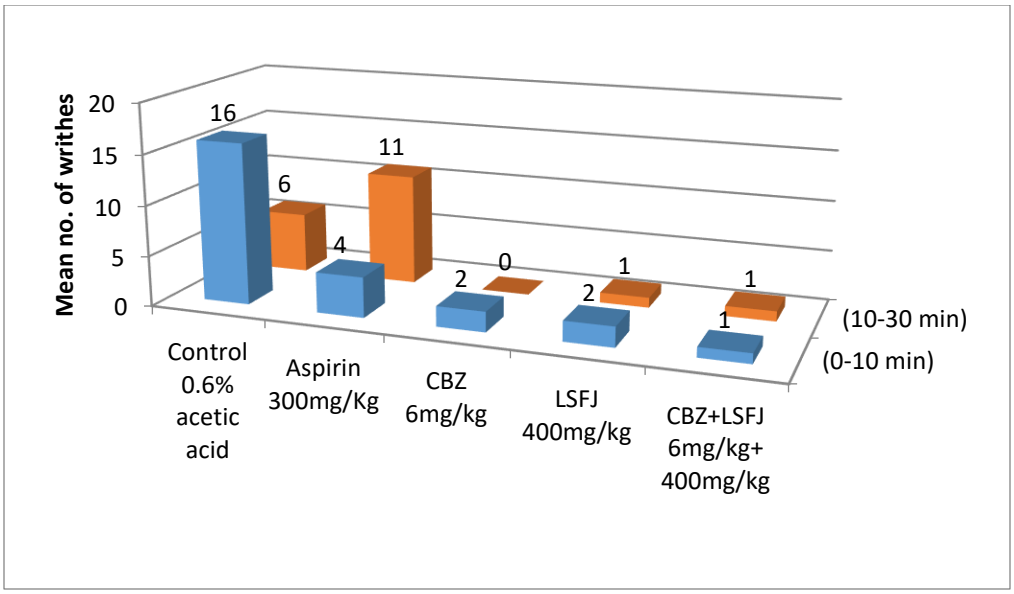


Figure - 1: Estimation of analgesic activity (acetic acid method) in control and treated animals. (mean±SEM). CBZ - Carbamezapine, LSFJ - *Lagenaria siceraria*

Anti-inflammatory activity

Formalin test

Results showed that *L. siceraria* reduced the licking and biting with high significance, however, the extent of effect was less than CBZ. Combination of CBZ and *L. siceraria* significantly reduce licking/biting as compared to the use of either CBZ or *L. siceraria* alone. It gives an evidence for the synergistic anti-inflammatory effect of this combination.

Table 3 Effect of Carbamezapine , *Lagenaria siceraria* fresh juice and their combination on anti-inflammatory activity

(formalin method).

Treatment	**Mean no. of licks/bites (0-10 min)**	**Mean no. of licks/bites (10-30 min)**
Control 2% formalin (hind paw)	29 ± 1.67	31 ± 1.06
Aspirin (300mg/Kg)	7 ± 0.73	3 ± 0.05
% inhibition	75.8%	90.3%
***p* value**	$p<0.0001$	$p<0.0001$
LSFJ (400mg/kg)	16 ± 1.31	11 ± 1.08
% inhibition	44.8%	64.5%

***p* value**	$p < 0.0014$	$p < 0.0010$
CBZ (6mg/kg)	12 ± 1.16	8 ± 1.28
% inhibition	58.6%	74.1%
***p* value**	$p < 0.025$	$p < 0.0004$
CBZ + LSFJ (6mg/kg/400mg/kg)	9 ± 1.27	7 ± 1.87
% inhibition	68.9%	77.4%
***p* value**	$p < 0.0067$	$p < 0.0054$
LSFJ – *Lagenaria siceraria*, CBZ – Carbamazepine. $p<0.05$ is significant ; $p<0.001$ highly significant Values are expressed in Mean ±SEM; n=5		

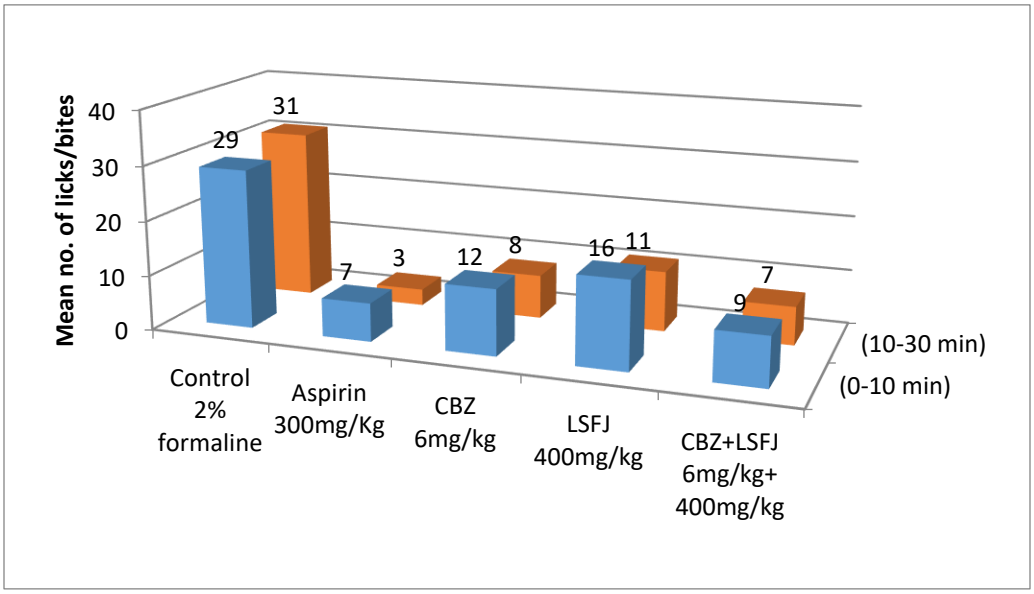


Figure - 2: Estimation of anti-inflammatory activity (formalin method) in control and treated animals. (mean±SEM). CBZ - Carbamezapine, LSFJ - *Lagenaria siceraria*.

Hind paw edema

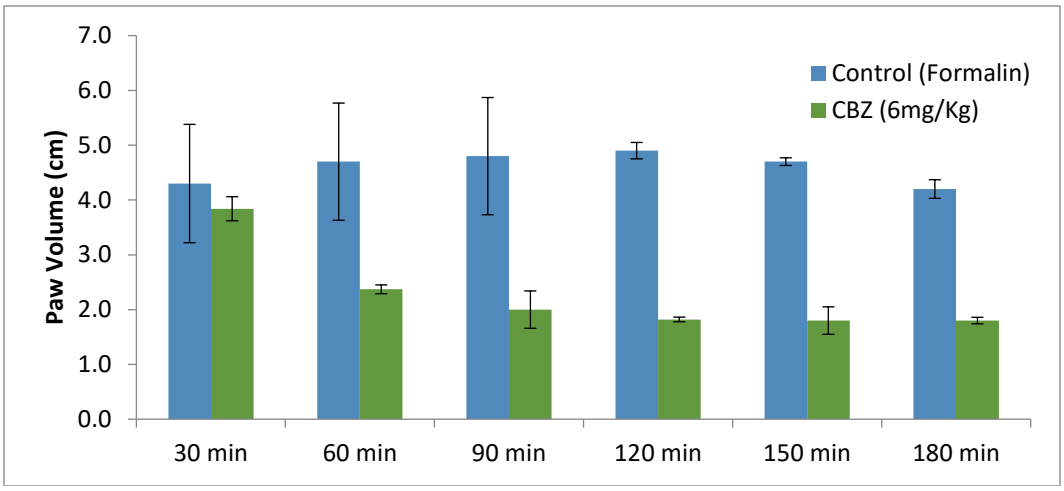
Results of hind paw volume followed by the injection of formalin are presented in Table - 4. Lesser swelling of paw was considered as the better control of inflammation. *L. siceraria* was not as effective in reducing the paw volume as CBZ but the combination of CBZ and *L. siceraria* minimized the swelling continuous during 3 hour of duration. Significant reduction in their combination showed possible synergism.

Table - 4 Effect of *Lagenaria siceraria* fresh juice, Carbamezapine and their combination on anti-infammatory activity (hind paw edema).

Treatment	Mean Hind Paw Volume (cm)						
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Control Saline	2.00±0.06	2.01±0.197	2.00±0.18	2.00±0.18	2.01±0.15	2.00±0.13	2.00±0.14
Control 2% w/v Formalin	2.20±0.12	4.3 ± 1.08	4.7 ± 1.07	4.8 ± 1.07	4.9 ± 0.15	4.7 ± 0.07	4.2 ± 0.17
Aspirin	2.10±0.25	3.12±0.058	3.12±0.03	2.9 ± 0.12	2.71±0.08	2.5 ± 0.04	2.4 ± 0.03
% inhibition	4.5%	27.4%	33.6%	39.5%	44.7%	48%	42.8%
LSFJ (400mg/Kg)	2.00±0.12	3.42 ± 0.58	3.12 ± 0.03	2.8 ± 0.26	2.53 ± 0.08	2.2 ± 0.05	2.2 ± 0.02
% inhibition	9%	20.5%	33.6%	41.6%	48.3%	53.1%	47.6%
CBZ (6mg/Kg)	1.90±0.12	3.84 ± 0.22	2.37 ± 0.08	2.0 ± 0.34	1.82 ± 0.043	1.8 ± 0.25	1.8 ± 0.06
% inhibition	13.6%	10.7%	49.5%	58.3%	61.8%	61.7%	57.1%
CBZ + LSFJ (6mg+400mg /Kg)	1.90±0.07	3.02 ± 0.14	2.46 ± 0.27	2.1 ± 0.17	2.06 ± 1.24	1.9 ± 0.03	1.9 ± 0.05
% inhibition	13.6%	29.7%	47.6%	56.2%	57.5%	59.5%	54.7%

LSFJ – *Lagenaria siceraria*, CBZ – Carbamazepine.
$p<0.05$ is significant ; $p<0.001$ highly significant
Values are expressed in Mean ±SEM; n=5

A



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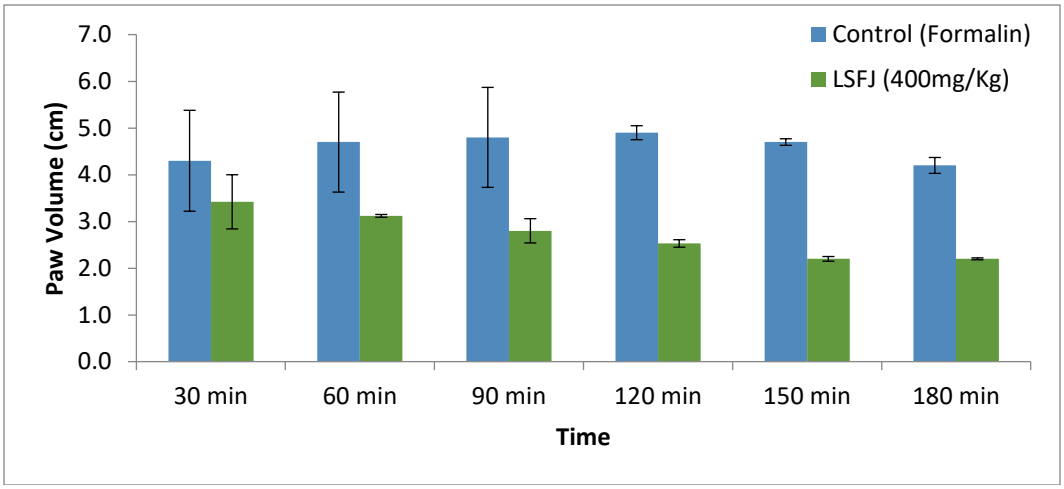

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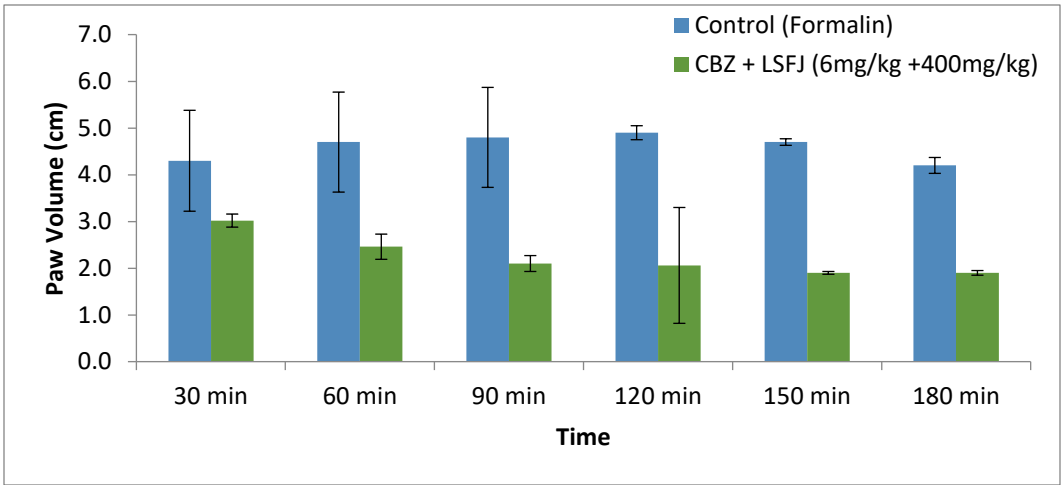

Figure 1: Anti-inflammatory activity (hind paw edema method) (CBZ - Carbamazepine, LSFJ - *Lagenaria siceraria's* fresh juice). A –Carbamazepine, B –*L. siceraria's fresh juice*, C –combination of Carbamazepine and *L. siceraria's* fresh juice

DISCUSSION

Anti-convulsion activity

200mg/kg dose of pilocarpine produced limbic motor seizures (Cavalheiro *et al.*, 1987, Turski *et al.*, 1983, Zhao *et al.*, 1994). After induction of pilocarpine the rats developed status epilepticus , starting its symptoms from stage 1 (tonic clonic) in which vertigo occurred followed by stage 2 body twitching, stage 3 in which seizures occurred and stage 4 which determined the survival or death.

First seizure occurred at 17.41 ± 0.24 min and 21.44 ± 0.70 min in control and *L. siceraria* treated group respectively. On the other hand no seizures were observed in CBZ and combination of CBZ+*L. siceraria* treated group, which exhibited antiepileptic effect of CBZ. *L. siceraria* may delayed the onset of convulsions and reduced the symptoms which proceed to seizures/convulsions but it did not inhibit the convulsion upon single dose administration. CBZ protected the onset of convulsion in combination group. Oxidative stress ultimately lead to the death of neurons, therefore, the use of antioxidants reduced oxidation (Kong and Lin, 2010). It might be possible that the regular use of *L. siceraria's* fresh juice may protect the occurrence of convulsions or seizure.

Analgesic activity

Analgesic activity is generally performed through the acetic acid induced abdominal constriction, which is one of the most common methods (Binder and Walker, 1998). Results showed that analgesic activity of CBZ and *L. siceraria* was significant in both phases (1st and 2nd) in comparison with standard drug aspirin (**Error! Reference source not found.** & **Error! Reference source not found.**). CBZ and *L. siceraria* showed 100% and 83.3% reduction in number of writhes respectively in neurogenic phase (1st Phase). While in licking and biting test CBZ and *L. siceraria* reduced pain up to (58.6% and 74.1%) and (44.8% and 64.5%) respectively, in both phases. It is most likely that, they might induced analgesia by inhibiting the production and release of prostaglandins which is a known mechanism of aspirin (Shah and Seth, 2010, Todorovic *et al.*, 2003). It may also possible that the active constituents of *L. siceraria* produced analgesic response. Further studies at molecular level may require to confirm its mechanism of action.

CBZ showed its analgesic effect by altering glutamate receptor activity besides blocking sodium channels (Jensen, 2002). In different types of epilepsy, cellular changes occurred due to the increased activity of glutamic receptors, atypical expression of Na^+ channels, changes in the inhibition of GABA receptors and an alteration in the entry of Ca^+ into the cells (Jensen, 2002). Combination of CBZ and *L. siceraria* also possessed significant analgesic activity in both tests, number of writhes and licking and biting test, which were more pronounced in neurogenic phase as compared to peripheral phase. These results showed that both drugs supported each

other to reduce pain beside their individual analgesic effect. Hence, could be effective in various pain disorders, especially *L. siceraria* which is a natural source of drug.

Anti-inflammatory activity

Inflammation is a defensive response of body immune system, often as a result due to infection. The main symptoms are redness, swelling and irritation. One of the cause of epilepsy is inflammation in the brain (Vezzani *et al.*, 2013). In hind paw edema test, CBZ reduced the inflammation up to 61.8% at 120 min during 3 hours of observation. CBZ might produce its anti-inflammatory action by inhibition of inflammatory mediators prostaglandin E2 and nitric oxide (Matoth *et al.*, 2000).

L. siceraria showed 53.1% reduction in inflammation at 150 min during 3 hour of observation. These results were similar to the effect of aspirin, which was used as a standard drug. Aspirin reduce edema by blocking cyclooxygenase and lipo-oxygenase pathway. The mechanism of anti-inflammatory action of *L. siceraria*, is not clear. However, by virtue of its antioxidant property, *L. siceraria* may limit the activity of overly produced nitric oxide (free radical), which involved in several immune responses, therefore exhibiting some reduction in inflammation and tissue damage (Palmer *et al.*, 1987, Rees *et al.*, 1989).

Synergistic anti-inflammatory effect was observed in a combination of CBZ+LSFJ treated group where 59.5% reduction in hind paw edema test was recorded at 150 min during 3 hours of reading.

Conclusion:

Fresh juice *of Lagenaria siceraria* processes anti-inflammatory and analgesic effects and the its use with carbamezapine does not alter its therapeutic effects *L. siceraria* can be a good food source for treatment inflammation and as a supportive medicine in convulsion.

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