CASSIA SIAMEA LAM EXTRACTS ANALGESIC MECHANISM OF ACTION AND PHARMACODYNAMIC INTERACTION WITH PARACETAMOL (ACETAMINOPHEN)

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ABSTRACT

Investigation of analgesic activity with oral administration of ethanol (CSE3) and aqueous (CES4) *Cassia siamea* extracts showed a dose-dependent profile similar with morphine in albinos Wistar rats. And ip injection of 1ml/kg of naloxone, a specific antagonist of morphine, 45 min after oral administration of extracts at the effective dose of 200 mg/kg completely reversed the analgesic effect. However, *C. siamea* extracts did not reverse Brewer'yeast-induced hyperthermia. The therapeutic combination of paracetamol with CSE3 or CSE4 extract, at doses of 8/15, 17/33 and 50/100 mg/kg p.o. (order: paracetamol/extract) exhibited significant and dose-dependent analgesic effect, higher than the effect observed with each product used alone at high dose. Pain was totally suppressed up to the limit fixed for experimental with association at dose (50/100) mg/kg. These observations indicate that *C. siamea* extracts do not target the cyclo-oxygenases of cerebral tissue as paracetamol do, but act through the CNS β mu opioid receptors as morphine. The synergic action of the extracts with paracetamol can be explained by a potentiation of the analgesic effect of these two drugs active each on a different target.

Keywords: Analgesic, *Cassia siamea*, mechanism, paracetamol, potentiation.

INTRODUCTION

Many public health problems are mainly caused by the toxicity, the loss of efficiency, the high cost and the side effects of the drug. Scientists use the introduction of therapy combination for the primary purpose of improving the overall efficiency and optimize the benefit/risk by reducing the dosage, but also of extending the duration of action, broadening the spectrum efficiency, reducing addiction, improving adherence and minimizing the risk of misuse.

Drug association is a combination of drugs which have similar pharmacological properties involved in a patho-physiologic identity, but acting simultaneously on different targets. It is

believed that morphine acts on the central nervous system (hypothalamus and limbic system) by saturation of opiate receptors mu (μ) subtype, involved in the phenomenon of pain perception. The action of morphine on opiates receptors in the rest of the body is causing side effects as constipation, respiratory depression, tolerance, dependence etc. Like some narcotics, morphine is a doping substance (Kleber al., 1994).

These side effects limit the use of this product which is the most effective on acute pain. It therefore becomes necessary to develop new analgesic drugs with the same mechanism of action than morphine, but without the most side effects of morphine, such as addiction, constipation and tolerance.

Paracetamol is well tolerated, when is taken in therapeutic doses **(**Martin et al**.,** 2004). Unlike opioids, paracetamol does not cause euphoria or change in mood. Paracetamol would have inhibitory action on the central nervous system in the prostaglandins production, involved in the process of pain and fever, through action on the prostaglandin H2 synthase (PGHS) enzyme. The latter includes an active site "cyclo-oxygenase" (or COX), target of most NSAIDs, and a peroxidase site (or POX). Which acts with the paracetamol (Tjolsen et al., 1991). In contrast to the NSAIDs those act on both isomorphic COX (COX-1 and COX-2 (Page et al., 2000), paracetamol has no direct action on COX-1 and COX-2(Jwiak-Benista and Nowak 2014). Indeed, paracetamol potentiates effect on serotonin descendants' neurons of the spinal cord and exerts control inhibitor of pain pathways. In addition, paracetamol could also act by limiting the release of beta-endorphins (Jwiak-Benista and Nowak 2014).
To take advantage of its analgesic and antipyretic properties and its tolerance, paracetamol is used in combination with other analgesic drugs to give a drug highly effective and better tolerated.

The previous pharmacological studies showed that *Cassia siamea* Lam (Fabaceae) is well tolerated (Mohammed et al., 2012) and possesses significant dose dependent analgesic and anti-inflammatory activities (Nsonde Ntandou et al., 2010 et 2015; Qiu-Fen et al., 2014; Mamadou et al., 2014). This study aims to determine the mechanism of action of the analgesic activity and assess the analgesic and antipyretic effect of interaction between paracetamol with each of these extracts.

EXPERIMENTAL

Plant material

Stem barks of *C. siamea Lam* were collected from Mindouli (Pool, Congo) in May 2007 and authenticated by the botanists of Centre d'Etudes sur les Ressources Végétales (CERVE), Brazzaville –Congo. A voucher specimen has been deposited at the Herbarium of the botanic laboratory.

Extraction

Dried and powdered stem barks were successively macerated 48 h at room temperature with petroleum ether, then dichloromethane, then ethanol. All organics extracts were concentrated to dryness under reduced pressure in a rotary evaporator to give the petroleum ether extract CSE1 (yield: 0.62%), the DCM extract CSE2 (0.92%) and the ethanol extract (0.80 %). The marc resulting from the extraction with ethanol has been extracted for 10 minutes in boiling water. The cold aqueous extract was centrifuged (7000 rpm during 30 minutes) and concentrated under reduced pressure with a rotavapor before lyophilization to give CSE4

(yield: 1.10%). CSE1 and CSE2 were solubilized in DMSO 2% while CSE3 and CSE4 were solubilized in distilled water before being fed to the rats.

Animals

Male and female Wistar rats (200-350 g) were obtained from the Health Science Faculty of Brazzaville for the experiments. They were housed under standard conditions (25 ± 5° C, 40-70 % RH, 12h light/dark cycle) and fed with a standard died and water ad libitum. The ethical rules published by International Association for the Study of Pain (Zimmermann, 1983) were respected.

Profiles dose/ analgesic effect curves in the hot plate test

Wistar rats were randomly assigned to nineteen groups of five animals each. The negative control group received distilled water (5 ml/kg) p.o. Plant extracts were given at the doses of 25, 50, 100, 200, 400 and 800 mg/kg p.o. from second to seventh group for CSE4, and then from eighth to thirteenth group for CSE3. Morphine which served as positive control was given p.o. at the doses of 1, 2, 4, 8, 18 and 32 mg/kg from ourteenth to nineteenth group. Analgesic activity was measured 1h after administration of test and standards drugs (Woolfe and McDonald 1994). Each rat was placed on the hot plate at 55 ± 0. 5°C (Ugo Basile DS-37) and the pharmacological activity was estimated by the measuring of latency period that precedes the animal reaction of licking its hind paw or jump.

Profiles dose / analgesic effect curves on the animal paw pressure

Wistar rats were randomly assigned to nineteen groups of five animals each. The negative control group received distilled water (5 ml/kg) p.o. CSE4 was given at the doses of 25, 50, 100, 200, 400 and 800 mg/kg p.o. from the second to the seventh groups respectively. CSE3 was given at the same conditions from eighth to thirteenth groups. Morphine which served as positive control was given at the doses of 1, 2, 4, 8, 18 and 32 mg/kg p.o. from the fourteenth to the nineteenth groups respectively. Analgesic activity on the animal paw pressure using an analgesimeter (Ugo basile 1740, Italy) was studied according to Randall and Sellitto 1957. Analgesic activity was measured 1h after administration of test and standards drugs (Abena et al., 2003). In rat subjected to pressure of the left leg (Elion Itou et al., 2014), we measured the intensity of the threshold pain that triggers the withdrawal of the animal paw and determined the reaction time.

Analgesic inhibition effect by naloxone in the hot plate test

The rats showing nociceptive response within 20 seconds when placed on the hot plate were selected, put into eight groups of five animals each. The first four groups received respectively p.o. distilled water (5 ml/kg), morphine (2 mg/kg), CSE4 or CSE3 (200mg/kg). The last four groups received the same treatments, but received also 45 min before an i.p. injection of naloxone (1ml/kg). The test was then performed as before with the study of profiles dose/ analgesic effect curves in the hot plate test.

Analgesic Inhibition effect by naloxone on the rat hind paw pressure

Rats were divided into eight groups of five animals each. The first four groups received respectively p.o. distilled water (5ml/kg), morphine (2 mg/kg), CSE3 (200 mg/kg) and CSE4

(200 mg/kg). The last four groups received the same treatments, and also 45 min before an i.p. injection of naloxone (1ml/kg). The test was then performed as before with the study of profiles dose / analgesic effect curves on the animal paw pressure.

Effect on yeast-induced pyrexia

Antipyretic studies test was studied by using the brewer's yeast-induced pyrexia according to Ahmed et al (1993). The test was performed in rats by the administration of a 20% aqueous suspension of brewer's yeast to induce pyrexia, at the dose of 20 ml/kg. Animals were then fasted for the duration of the study (approx.24hours), but water was made available ad libitum. Rectal temperature of each animal was taken before and 24 hours after the yeast administration using digital clinical thermometer (Hartmann, Germany). Animals that did not show a minimum increase of 0.5°C in temperature 24 hours after yeast injection was discarded. The selected animals were grouped into twelve groups of five animals each. The first two groups of control received distilled water (5 ml/kg) or DMSO 2 % (5 ml/kg) p.o. The positive control group received paracetamol (50 mg/kg p.o.) and the test groups received CSE1, CSE2, CSE3 or CSE4 at the doses of 200 and 400 mg/kg p.o. The rectal temperature of each animal was again recorded at 0; 0.5; 1; 2; 3 hours after treatment. Antipyretic effect was rated as the ability of the test to reverse the induced pyrexia.

Potentiating of analgesic effect by paracetamol/plant extract associations in the hot plate test

Selected rats were divided into twelve groups of five animals each. The negative control group received p.o distilled water (5 ml/kg), of distilled water. The positive control group received p.o. paracetamol (50 mg/kg). CSE4 was given at the doses of 100 and 200mg/kg p.o. to the third and fourth groups. CSE3 was given at the doses of 100 and 200mg/kg p.o., to the fifth and sixth groups. The combination of paracetamol with CSE4, at doses of (8/15), (17/33) and (50/100) mg / kg p.o. (order: paracetamol/extract), was given to groups 7, 8 and 9 while groups 10, 11 and 12 received the combination of paracetamol with CSE3 at the same conditions that precedently. The test was then performed as described by Woolfe and McDonald 1994.

Potentiating of analgesic effect by associations on the animal paw pressure

Rats were divided to twelve groups of five animals each. A control group received 5ml/kg, p.o., of distilled water. Paracetamol (50mg/kg, p.o.). Paracetamol was given to second group which served as standard. *C. siamea* aqueous extract were given at the doses of 100 and 200mg/kg p.o., to the third and fourth groups. *C. siamea* ethanol extract were given at the doses of 100 and 200mg/kg p.o., to the fifth and sixth groups. The combination of paracetamol with aqueous extract, at doses of (8/15), (17/33) and (50/100) mg / kg p.o. (order: paracetamol/extract), was given to groups 7, 8 and 9; while groups 10, 11 and 12 have been treated by the combination of paracetamol with CSE3, at seems doses. The test was then performed as described by Nsonde Ntandou et al. (2015).

Analgesic effect on time reaction on the animal paw pressure

The control groups received distilled water (5 ml/kg) p.o., ethanol and aqueous extracts were given at the dose of 200 mg/kg p.o. Paracetamol which served as a standard was given at the dose of 50mg/kg, p.o. Associations of paracetamol and CSE3 or CSE4 were given at the dose

of (50/200)mg/kg), in order: paracetamol/ extract. Effect measures were made 5min, 15min, 30min, 60min, 90min, 120min, 150 min and 180 min. In each experience, rats' lots have been renewed.

RESULTS

Profiles dose/ analgesic curves effect

Both extracts and morphine showed dose dependent analgesic effect with similar profiles (figures: 1, 2a and 2b).

Analgesic inhibition effect by naloxone

Naloxone exhibited significant reduction of threshold intensity and time reaction in a control group ($p< 0, 05$). In the treated groups the analgesic effect was completely reversed by naloxone (figures: 3, 4a and 4b).

Effect on yeast-induced pyrexia

The result showed that only paracetamol and paracetamol/plant extracts associations significantly reversed hyperpyrexia in rats (figure: 5) and the effect was not different between paracetamol and its association with extracts.

Potentiating of analgesic effect by plant extracts/paracetamol combinations

Pain was totally suppressed by the administration of analgesic combination (figures: 6a, 6b and 7), up to the limit fixed for experimentation with association of 50/100 mg/kg for both extracts.

Effects of analgesic combinations on reaction time

The result of analgesic effect on time reaction study is showed in figure: 8a and 8b. The maximum analgesic effect of simple product and association was obtained at the same moment, from 15min to 75 min after oral administration and 90 min after the effect disappeared. The effect of association was most important follow by the effect of aqueous extract (CSE 3).

DISCUSSION

Hot plate and hind paw pressure tests confirmed the analgesic effect already observed in this plant or in the other species of the genus (Nsonde et al., 2010 and 2015; Palanichamy and Nagarajan, 1990; Jain et al., 1997; Chidume et al., 2002; Adzu et al, 2003) and permitted to elucidate part of the mechanism of action. The similarity of profiles dose/ analgesic effect for the aqueous and ethanol extracts of *Cassia siamea* stem bark indicates that they share probably the same active compound(s), which could have the same mechanism of action than morphine. We confirmed these hypotheses by verifying if the analgesic effect was reversed by naloxone, a well-known.specific inhibitor of morphine. Since naloxone completely reversed *C. siamea* analgesic effect, there is strong probability that the active compound(s) in *C. siamea* extracts target the central nervous receptors βμ, as morphine does (Morundee et al., 2002).

In addition, we know that the antipyretic effect of paracetamol is due to his action on the cyclo-oxygenase of the cerebral tissue (Cannon et al., 2001). To verify if *C. siamea* extracts could also target the cyclo-oxygenases, we studied the antipyretic effect of the extracts and of their associations with paracetamol. The absence of antipyretic effect from extracts and the

absence of potentiating of its effect by association indicate that *Cassia siamea* extracts do not use the same mechanism of action than paracetamol. We investigated the pharmacodynamic interaction between *Cassia siamea* extracts and paracetamol since we had demonstrated that the plant extracts potentiated the analgesic action of paracetamol. This interaction could be explained by a synergic action of associated products mainly the triterpenoïds as lupeol (Lucetti et al., 2010; Ogbole et al., 2014).

Generally, the effect of a drug persists as long as the drug or its active metabolite is present and available in the body in sufficient concentration. This period has pharmacokinetics origin and corresponds to the time needed for the drug to reach its targets. The pharmacokinetic profiles of paracetamol and that of *Cassia siamea* extract are similar. Extracts given orally are absorbed easily and their maximum active plasma concentration is reached after 15 minutes and remains available and sufficient for about 90 minutes. It was shown that the orally administered paracetamol reaches its maximum plasma concentration approximately 30 minutes for duration of about 2 hours in humans. There is no pharmacokinetic interaction between paracetamol and extracts, since the profiles of the analgesic combinations plant extract/paracetamol are exactly the same as the profile of paracetamol alone

CONCLUSION

On the basis of the results in this study, it can be concluded that the mechanism of analgesic effect of *C. siamea* Lam stem bark aqueous and ethanol extracts is identical to morphine. As morphine *C. siamea* extracts act on the central nervous through receptors βμ. *C. siamea* extracts used in association develop a pharmacodynamic synergic analgesic effect with paracetamol. C.siamea extracts and its association can be used as analgesic in acute and subchronic pain.

ACKNOWLEDGMENTS

The authors are grateful botanists of Laboratoire de Botanique du Centre d'Etudes sur les Ressources Végétales, Brazzaville-Congo for authenticating the plant materiel and animal house technicians of Faculté des Sciences de la Santé of Université Marien Ngouabi of Brazzaville- Congo for the maintenance of animals.

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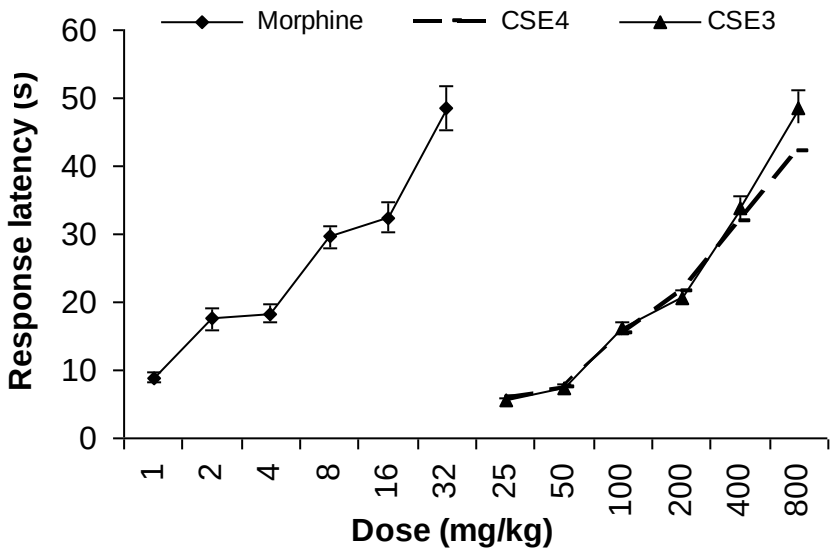
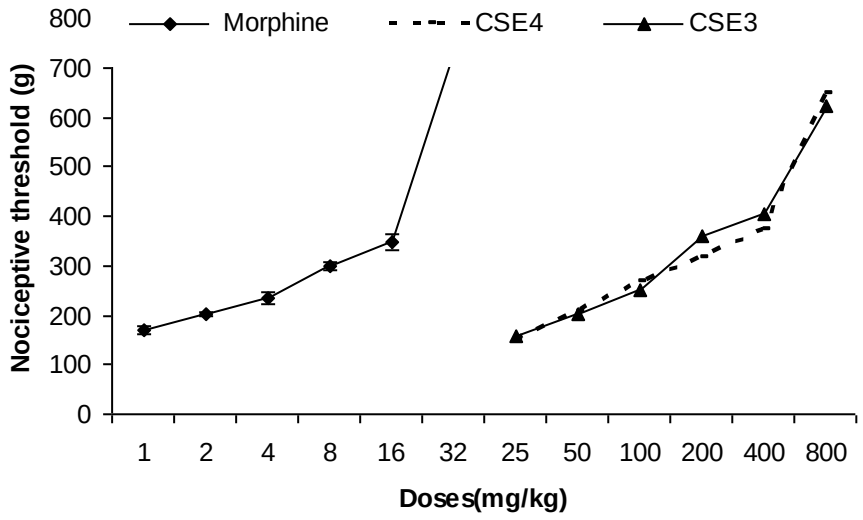
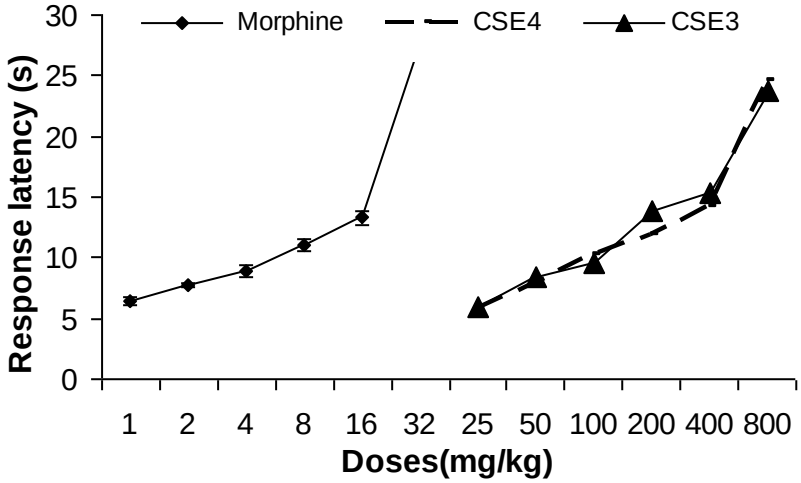


Figure 1: Similar profiles dose/analgesic effect of *C. siamea* stem bark extracts in rat using hot plate test

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract



(a)



(b)

Figure 2(a and b): Similar profiles dose/analgesic effect of *C. siamea* stem bark extracts on the rat paw pressure

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract

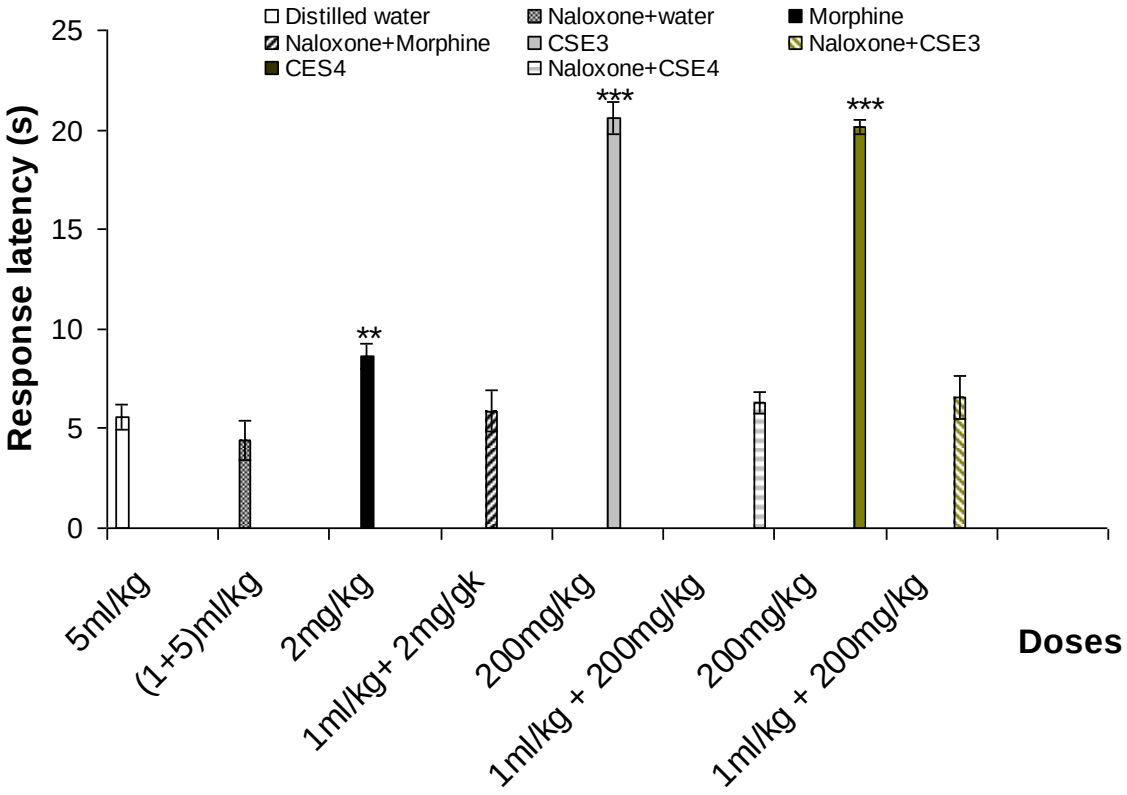
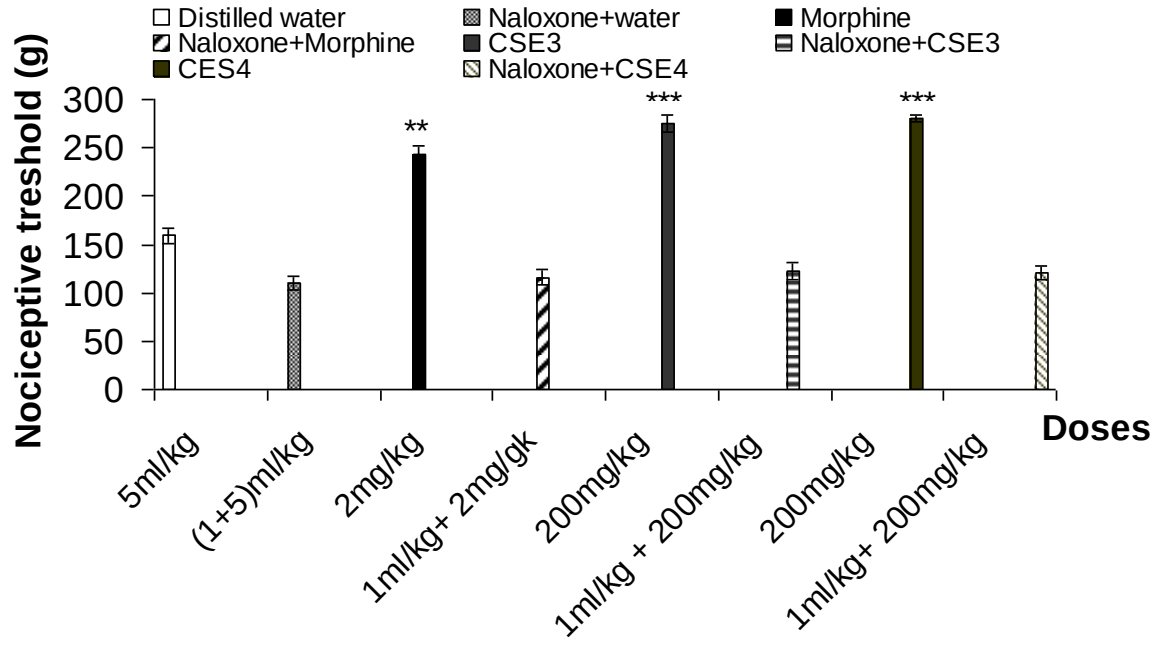


Figure 3: Inhibition of *C. siamea* stem bark extracts analgesic effect by nalaxone in rat using hot plate test

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract



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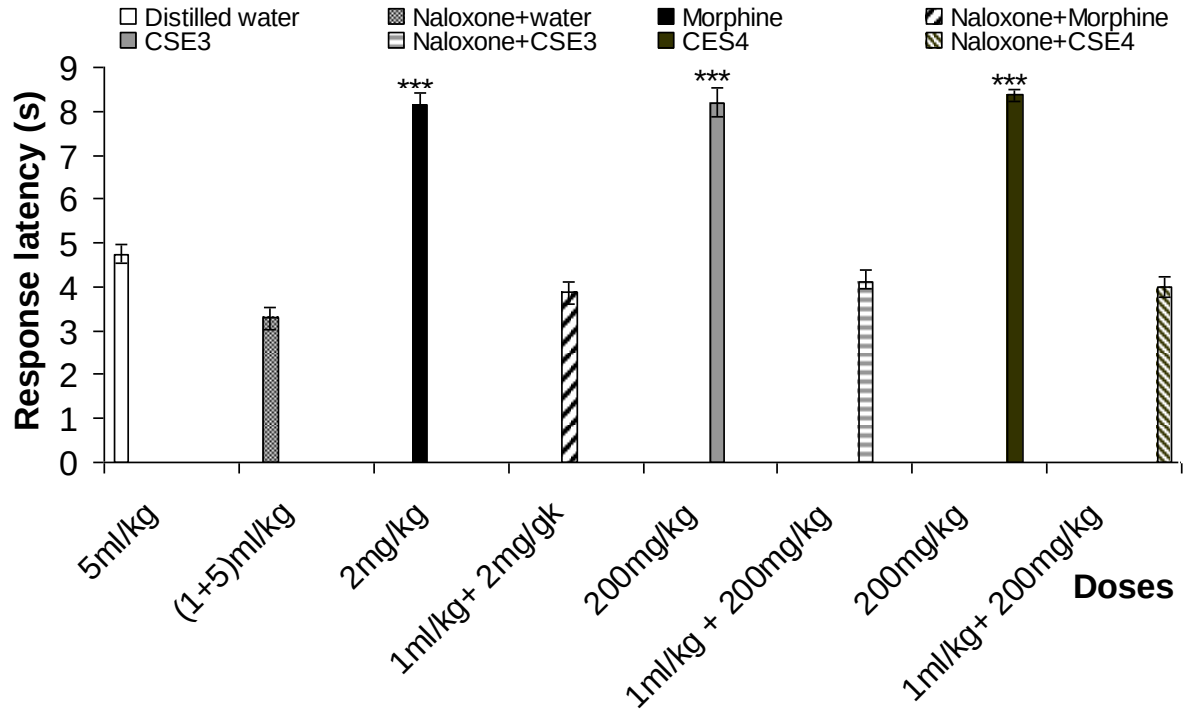


b)

Figure 4(a and b) : Inhibition of *C. siamea* stem bark extracts analgesic effect by nalaxone on the rat using paw pressure

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract

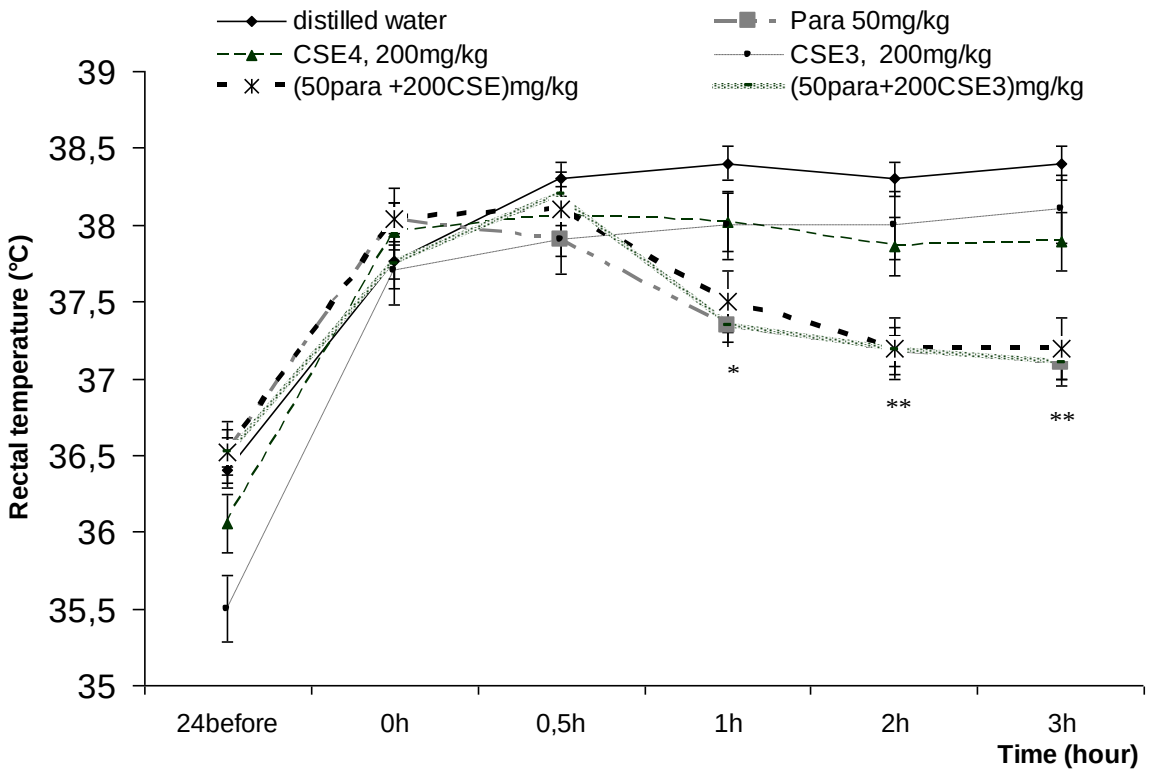
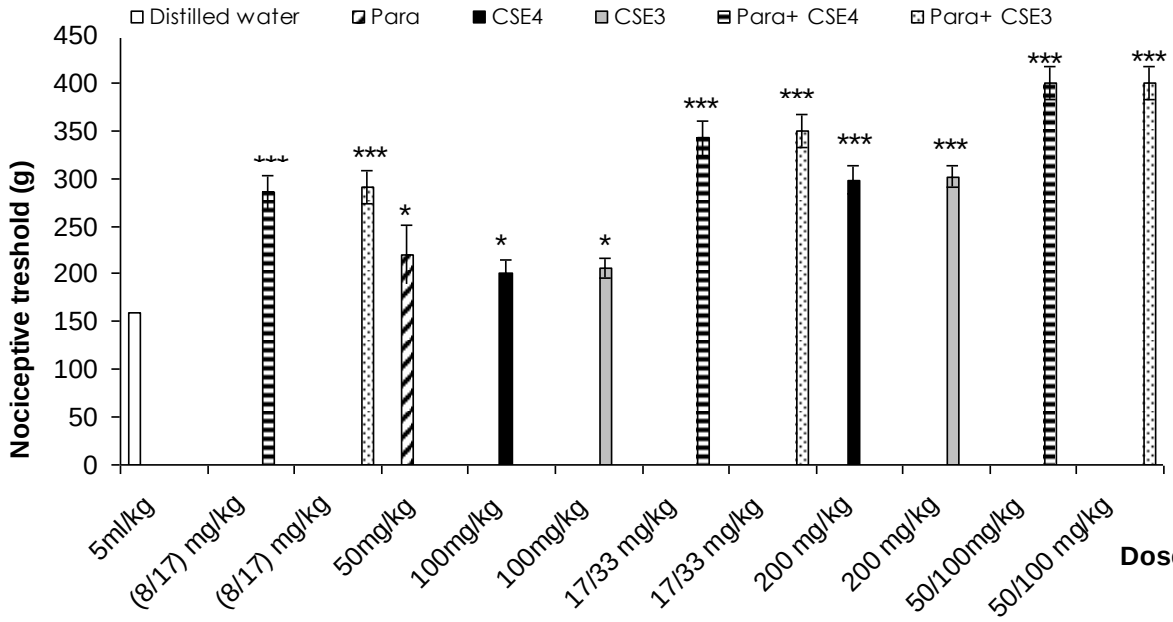
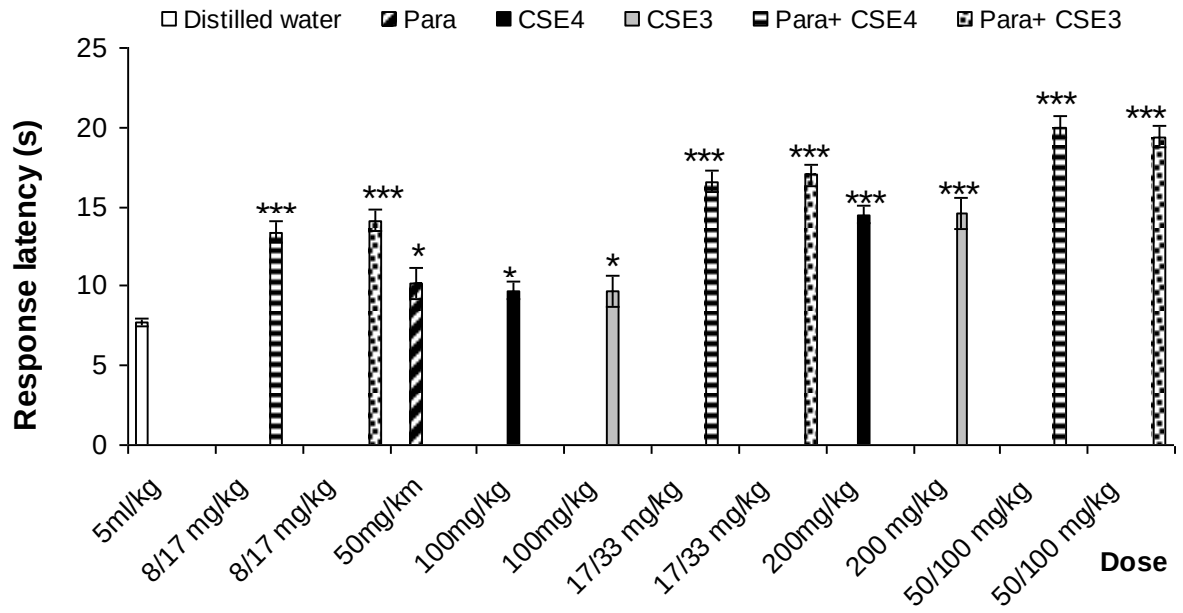


Figure 5: Absence of pharmacodynamic interaction on antipyretic effect of combination *C. siamea* stem bark extracts with paracetamol on brewer's yeast-induced pyrexia

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract



(a)



(b)

Figure 6 (a and b): Potentiate of analgesic effect by a combination of *C. siamea* stem bark extracts with paracetamol on the rat paw pressure

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract

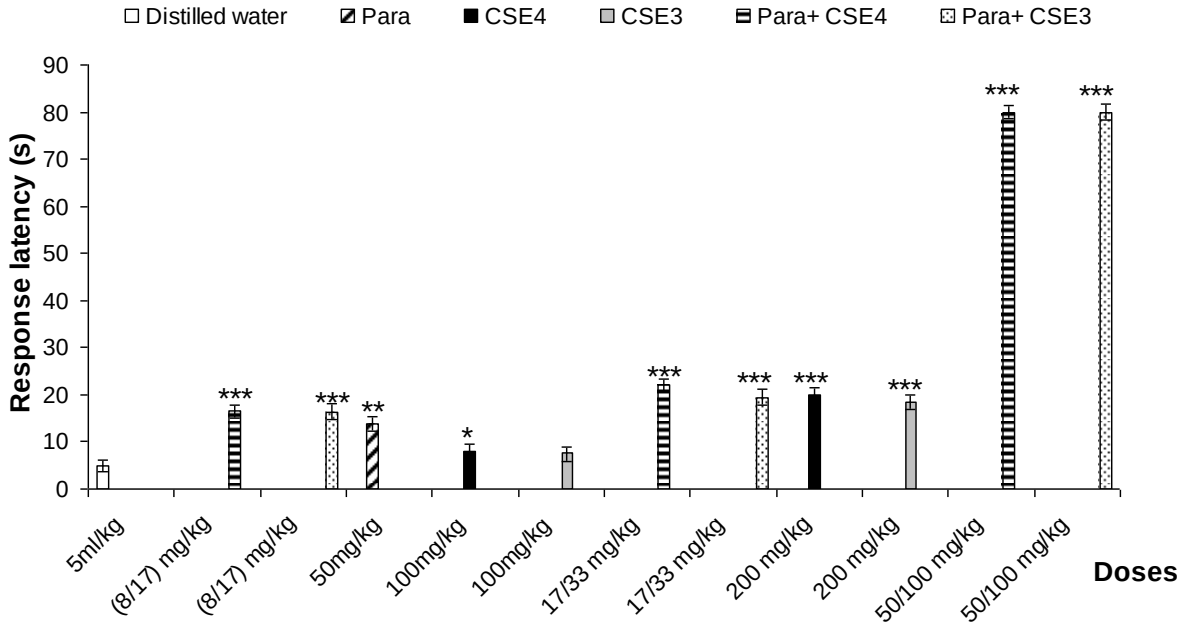
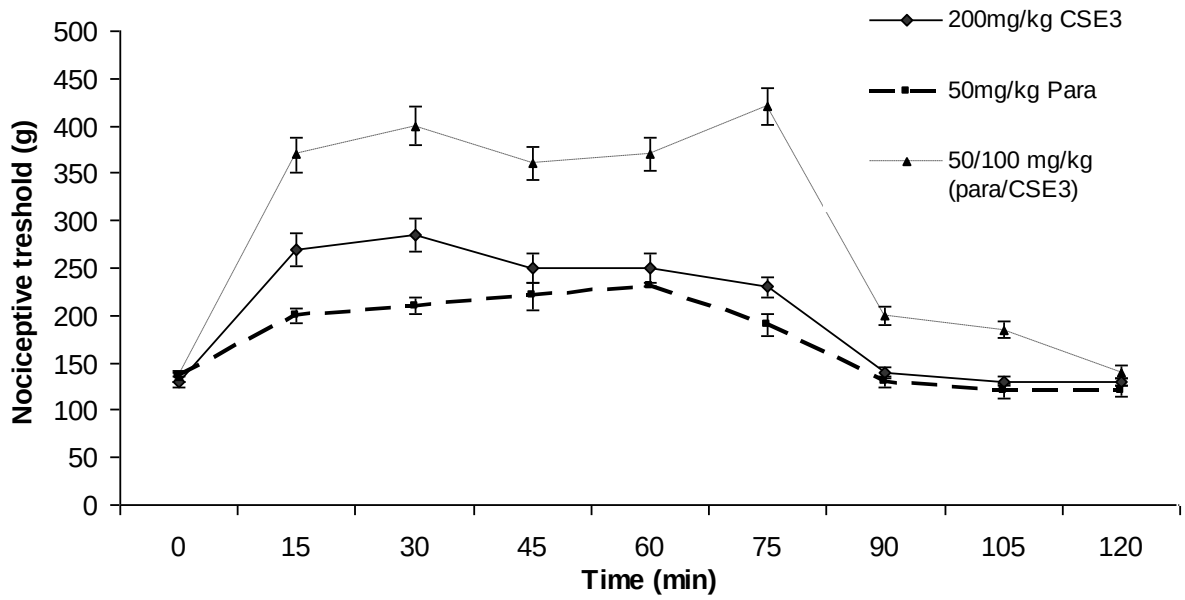
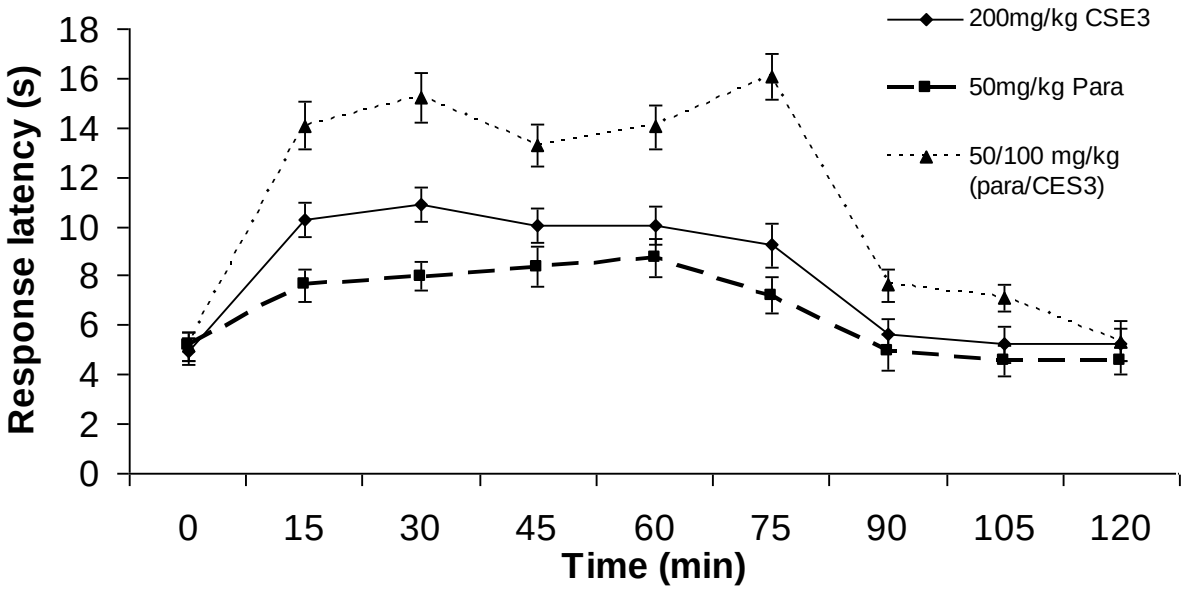


Figure 7: Potentiate of analgesic effect by a combination of *C. siamea* stem bark extracts with paracetamol in rat using hot plate test

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract



(a)



(b)

Figure 8 (a and b): Absence of analgesic interaction effect on time reaction by a combination of *C. siamea* stem bark extracts with paracetamol on the rat paw pressure

Results are expressed as means +/- S.D.; n=5; * p<0.5; ** p<0.01; *** p< 0.001 compared with control. CSE3: *C. siamea* stem bark aqueous extract; CSE4: *C. siamea* stem bark aqueous extract