International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 2 (1): 42-44, 2006
ISSN 1811-7775

Comparative Study of Analgesic Activities of Tétra® and an Association of Three Plants: *Ageratum conyzoides*, *Combopogon citratus* and *Lippia multiflora*

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Abstract: Aqueous extract of an association of *Ageratum conyzoides* (Ac), *Combopogon citratus* (Cc) and *Lippia multiflora* (Lm) produced a significant reduction in mouse of writhings induced by acetic acid and an increase of pain threshold in the hot plate test in mice. These effects were similar to that produced by the traditional improved preparation Tetra® which is prepared with twenty plants, among them, Ac, Cc and Lm. On contrary of morphine, analgesic activities of the extract association and Tétra® is not inhibited by naloxone. Chemical study revealed the presence in the two preparations of saponins and flavonoids which could support the observed activities. These results suggests that, this association of three plants could be used as traditional improved preparation in the aim to minimise the important number of plants in Tétra®.

Key words: Tétra®, association *Ageratum conyzoides*, *Combopogon citratus*, *Lippia multiflora*, analgesic, comparison

INTRODUCTION

Tétra® is a traditional improved preparation used by Congolese populations for the treatment of various diseases such as arterial hypertension, pectic ulcer, fever, inflammation and pain. Some pharmacological and clinical studies[1-4] have revealed hepatoprotective, analgesic, anti-inflammatory and anti-ulcer effects of this traditional preparation.

Tétra® is prepared with twenty plants , among them, *Ageratum conyzoides* (AC), *Combopogon citratus* (Cc) and *Lippia multiflora* (Lm). Many pharmacological studies[5-9] have demonstrated the analgesic properties of Ac and Lm. These analgesic effects could support the use of Tétra® in pain.

The objective of present study was to compare analgesic effect of Tétra® and those of an preparation which associate Ac, Cc and Lm in the aim to obtain another traditional improved preparation indicated in pain with minimum of three plants.

MATERIALS AND METHODS

Preparation of plants extracts: Fresh leaves of Ac, Cc, Lm were collected or purchased in Brazzaville area and authentified by botanists of Centre d'Etudes sur les Ressources Végétales.

The fresh leaves of the three plants (20 g of each plant) were mixed and boiling in 450 mL of distilled water for 30 min , decanted and filtered. The filtrate which was preserved at 4°C, constitute the association extract. Tétra® was obtained from local pharmaceutical market.

Acute toxicity: Mice were administered orally with extract 200- 300 mg kg^{-1}. Mortality in each group was observed for 24 h.

Acetic acid induced writhings test: Distilled water (5 mL kg^{-1}) for control group, Tétra® (0.5 mL kg^{-1}), aqueous extract of association Ac-Cc-Lm (200 mg kg^{-1}) or paracetamol (50 mg kg^{-1}) were orally administered to mice before intraperitoneal injection of acetic acid (0.6%, v/v in distilled water, 10 mL kg^{-1})[10]. The number of writhings exhibited by each animal was counted for 10 min beginning 10 min after acetic acid injection.

Hot plate test: The method already described by Hi kino *et al.*[11] was used with a slight modification. Mice were placed on a hot plate (56±1°C) and the reaction time (licking the paw) to thermal stimulus was observed with

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A cutt-off of 60 sec. Distilled water (5 mL kg^{-1}) for control group, Tétra® (0.5 mL kg^{-}), aqueous extract of association Ac-Cc-Lm (200 mg kg^{-1}) or morphine (Muscotin®, 2 mg kg^{-1}), were orally administered 1 h before the test.

Study of mechanism of analgesic effect: Fifteen min after intraperitoneal injection of naloxone (Narcan®, 1 mg kg^{-1}), Mice were treated and placed on a hot plate and the reaction time was determined as above described.

Chemical study: Classical phytochemical tests were used to identify the major chemical groups of Tétra® and association extract.

Statistical analysis: Results are expressed as mean±SEM. Student's t-test was used to analyse the significance of the results.

RESULTS AND DISCUSSION

Aqueous extract of association Ac-Cc-Lm is well orally tolerated by animal; until 3600 mg kg^{-1}, no mortality was observed. This extract, at the dose of 200 mg kg^{-1}, caused a significant ($p<0.001$) inhibition of control writhings (Table 1). This inhibition was similar to that produced by 200 mg kg^{-1} of Tétra® and 50 mg kg^{-1} paracetamol (- 68.90, -69.51 and -68.58%, respectively).

The extract increased significantly ($p<0.001$) the reaction time in the hot plate test (Table 2). The increase of reaction time exhibited by 200 mg kg^{-1} of extract was same as Tétra® 200 mg kg^{-1} and less than morphine 2 mg kg^{-1}. The reduction of acetic acid induced writhings and the increase in the reaction time to the thermal stimulus, suggest that the analgesic effect of extract and those of Tétra® are mediated both peripherally and centrally[12].

Table 1: Effects of Tétra® and association of Ageratum conyzoïdes, Combopogon citratus and Lippia multiflora on acetic acid induced writhings test

Products	Doses (PO)	Number of writhings	% inhibition
Control	5 mL kg^{-1}	65.60 ± 6.07	-
Paracétamol	50 mg kg^{-1}	20.60 ± 2.03***	68.58
Tétra®	0.5 mL kg^{-1}	20.20 ± 1.12***	69.51
A.E. « AC-Cc-Lm »	200 mg kg^{-1}	20.40 ± 1.12***	68.90

Table 2: Effects of Tétra® and association of Ageratum conyzoïdes, Combopogon citratus and Lippia multiflora on reaction time in the hot plate test

Products	Doses (PO)	Reaction time (sec)
Control	5 mL kg^{-1}	5.20 ± 1.31
Morphine	2 mg kg^{-1}	56.60 ± 5.29***
Tétra®	0.5 mL kg^{-1}	32.60 ± 8.02***
A.E. « AC-Cc-Lm »	200 mg kg^{-1}	33.40 ± 5.07***

A.E. « AC-Cc-Lm » :aqueous extract of association of *Ageratum conyzoïdes*, *Combopogon citratus* and *Lippia multiflora*, *** : $p<0.001$

Table 3: Effects of Tétra® and association of Ageratum conyzoïdes, Combopogon citratus and Lippia multiflora on reaction time in the hot plate test in the presence of naloxone

Products	Doses (PO)	Temps de reaction (sec)
Morphine+Naloxone	2 mg kg^{-1} + 1 mg kg^{-1}	6.80 ± 0.86***
Tétra®+Naloxone	0.5 mL kg^{-1} + 1 mg kg^{-1}	44.20 ± 7.51***
A.E. AC+Cc+Lm »+ Naloxone	200 mg kg^{-1} + 1 mg kg^{-1}	31.40 ± 3.44***

A.E. AC+Cc+Lm »: aqueous extract of association of *Ageratum conyzoïdes*, *Combopogon citratus* and *Lippia multiflora*, *** : $p<0.001$

Table 4: Phytochemical screening

Aqueous extract	1	2	3	4	5	6	7	8	9	10
Tétra®	±	+	-	+	-	+	+	-	+	+
Association Ac+Lm+Cc	++	+	±	-	±	-	+	-	±	+

1: Saponins 2: Flavonoïds 3: Alkaloïds 4: Terpénoïds/Stéroïds
5: Quinones 6: Polyphénols 7: Tanins 8: Anthocyans
9: Amino acids 10: Glucides

On the contrary of morphine 2 mg kg^{-1}, analgesic effects of association extract and Tétra® were not influenced by naloxone administration (Table 3), suggesting that these activities are not mediated by opioid receptors.

Chemical study (Table 4) revealed the presence of saponins and flavonoids in the two preparations. These chemical constituents could contribute to the observed analgesic activity.

These results suggests the possibility of the use of this association of three plants as traditional improved preparation in the treatment of pain.

REFERENCES

1. Abena, A.A., F.R. Boutété, H. Ntsiba, G.B. Ngouoni, M. Diatéwa and Th. Hondi-Assah, 2002. Etude clinique d'un phytomédicament congolais: le Tétra®. Phytothérapie, 19: 5-9.
2. Makambila-Koubemba, M.C., A.A. Abena and M. Ndounga, 1997. Activité antalgique d'un extrait brut d'*Ageratum conyzoides* chez la souris. Etude comparative au Tétra®. Pharm. Med. Trad. Afr., 9: 34-39.
3. Hondi-Assah, T.C., A.A. Abena, J. Kokolo, C. Badila and M. Diatéwa, 2003. Effets hépatoprotecteurs de Lippia multiflora et d'un phytomédicament congolais: le Tétra®. Phytothérapie, 5: 135-140.
4. Abena, A.A., J.F. Péko, G. Bazéka, T.C. Hondi-Assah and M. Diatéwa, 2003. Etude de l'effet anti-ulcereux de l'Ageratum conyzoides.Ann. Univ. M. Ngouabi, 4: 139-147.
5. Abena, A.A., G.S. Kintsangoula-Mbaya, J. Diantama and D. Bioka, 1993. Effets analgésiques d'un extrait brut d'*Ageratum conyzoides* chez le rat. Encephale, 19: 329-332.

6. Abena, A.A., D. Bioka, C. Samba-Badila, Th. Hondi-Assah and M. Diatéwa, 1997. Propiétés tranquillisante et antalgique du *Lippia multiflora*. Pharm. Med. Trad. Afr., 9: 94-107.
7. Abena, A.A., G.R. Ngondzo-Kombéti and D. Bioka, 1998. Propiétés psychopharmacologiques du *Lippia multiflora*. Encéphale, 24: 449-454.
8. Abena, A.A., J.M. Ouamba and A. Kéita, 1996. Anti-inflammatory, analgesic and antipyretic activities of essential oil of *Ageratum conyzoides*. Phytother. Res., 10: S164-S165.
9. Abena, A.A., M. Diatéwa, G. Gakosso, G. Gbeassor, Th. Hondi-Assah and J.M. Ouamba, 2003. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Fitoterapia, 74: 231-236.
10. Koster, R., M. Anderson and E.J. De Beer, 1959. Acetic acid for analgesic screening. Fed. Proc., 18: 412.
11. Hikino, H., K. Ogata, Y. Kasahara and C. Konno, 1985. Pharmacology of ephedroxanes. J. Ethnopharmacol., 13: 175-191.
12. Adeyemi, O.O., S.O. Okpo and O.O. Ogunti, 2002. Analgesic and anti-inflammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia, 73: 375-380.