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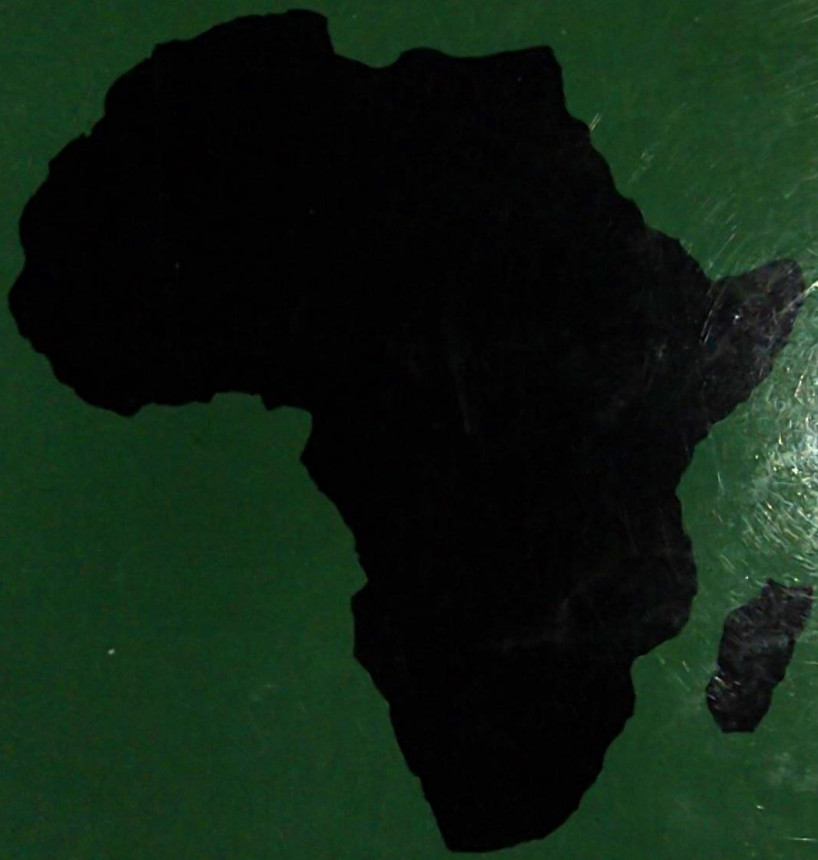

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Analgesic and anti-inflammatory properties of methanol extract of *cnestis ferruginea* in rodents

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Abstract

Objective: To evaluate the analgesic and anti-inflammatory activities of the methanol extract of *Cnestis ferruginea* (CF) in rodents.

Materials and Methods: The antinociceptive activity of CF was evaluated using thermal (hot plate and tail flick tests) and chemical (acetic acid induced writhing and formalin tests) methods. The anti-inflammatory effects were studied using the cotton pellet granuloma and carrageenan induced paw edema tests.

Results: The extract (300-500mg/kg, per oral), dissolved in normal saline produced a dose dependent analgesic effect on a hot plate maintained at 55 ± 2°C as well as on the early and late phases of formalin induced paw licking in rats. At the same doses, the plant extract also significantly ($p<0.05$) inhibited the carrageenan induced paw edema and cotton pellet granuloma formation in rats.

Conclusion: The results suggest that the methanol extract of CF possesses analgesic and anti-inflammatory activities.

Keywords: *Cnestis ferruginea, methanol extract, analgesic, anti-inflammatory*

Résumé

Objectif: Evaluer les activités analgésiques et anti-inflammatoires de l'extrait au méthanol de Cnestis ferrugineux (FC) chez les rongeurs.

Matériels et méthodes: L'activité anti nociceptive de la CF a été évaluée à l'aide de méthode thermique (plaque chauffante et des tests de mouvement de la queue) et chimique (acide acétique induite en contorsion et des essais de formol). Les effets anti-inflammatoires ont été étudiés en utilisant les tests de granulome des boulettes de coton et de patte œdème induite de carragheen.

Résultats: L'extrait (300-500mg/kg, par voie orale), dissous dans une solution saline normale a produit un effet analgésique à dose dépendant sur une plaque chaude maintenue à 55 ± 2 ° C ainsi que sur les phases précoces et tardives de la patte induite par le formol raclé chez des rat. Aux mêmes doses, l'extrait végétal aussi a significativement ($p <0,05$) inhibé l'œdème de patte induite par la carragheen et la formation de granulome de boulette de coton chez des rats.

Conclusion: Les résultats suggèrent que l'extrait de méthanol de CF possède des activités analgésiques et anti-inflammatoires.

Introduction

Cnestis ferruginea (Comaraceae) is a common plant found throughout the West African sub-region. It is a tree of about 6m high usually found in the deciduous forest. The plant has been shown to have an ornamental value [1] and its water extract used to treat various types of pain including tooth ache [2].. Some authors have also reported its effects on fertility and haematological profiles [3,4]. Phytochemical screening had confirmed the presence of flavonoids, alkaloids saponins and anti-oxidants [5,6]. Although the plant had been in use for a long time as herbal medicine in Nigeria, no pharmacological screening has been carried out to justify its use by the alternate medical practitioners as an analgesic and anti-inflammatory agent. In the present study, we therefore investigated these properties of the plant to justify or ortherwise the claim by the herbal practitioners.

Materials and method

Animals

Male albino rats (180-220g) and mice (50-80g) were used for the study. They were housed and bred in the preclinical animal house of the College of Medicine, University of Ibadan, Nigeria under standard laboratory conditions (room temperature and 12hours light and dark cycle) . The animals were fed with mouse cubes (Ladokun feeds, Ibadan) and water *ad libitum*

Plant materials

Fresh leaves of the plant were obtained from the Forestry Research Institute of Nigeria, Ibadan where the institute's plant taxonomist carried out the identification (Voucher no F 4137). The leaves, shade dried and reduced to a powdery form were exhaustively extracted at a temperature of 40°C with 2.5L of methanol (BDH Poole, England) for 3 days. The macerated mixture was filtered and evaporated

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Carrageenan induced paw edema

The anti-inflammatory potency of the CF extract is as shown in table 4. The extract significantly ($p<0.05$) inhibited edema formation only at the 500mg/kg dose level at 3 and 5hr post administration. The reference drug (indomethacin, 10mg/kg) also produced a significant ($p<0.05$) inhibition of edema formation compared with the control (Table 4).

[5,6].These agents have also been found in other plants with established analgesic and anti-inflammatory activities [15]. The extract reduced the licking time in both phases of the formalin test which is one of the tests commonly used to explain the mechanisms in pain and analgesia and it gives better results than thermal or mechanical tests [16].

The formalin test comprises two phases, the first representing the irritating effect of formalin on

Table 4: The effect of methanolic extract of Cnestis ferruginea on paw circumference in the carrageenan edema test

Groups	0 hr (cm)	3rdhr (cm)	Percent inhibition	5thhr (cm)	Percent inhibition
Control (normal saline)	2.13 ±0.05	2.82 ± 0.06	-	2.63 ± 0.02	-
300mg/kg *Cnestis feruginea*	2.25 ± 0.05	2.73 ± 0.06	30.40	2.57 ± 0.03^{NS}	36.00
400mg/kg *Cnestis ferruginea*	2-20 ± 0.04	2.63 ± 0.08^{NS}	37.68	2.42 ± 0.06^{NS}	56.00
500mg/kg *Cnestis ferruginea*	2.15 ± 0.07	2.53 ± 0.09*	44.92	2.37 ± 0.09*	58.00
10mg/kg indomethacin	2.17 ± 0.05	2.48 ± 0.04*	55.07	2.35 ± 0.04*	64.00

Each value is the mean ±SEM n=6
**P ≤ 0.05 compared with control*
NS not significant compared with control

Table 5: The effect of the methanolic extract of Cnestis ferruginea on granuloma tissue formation in rats

Groups	Oral dose	Increase in pellet weight (mg)	Percent inhibition
Control (normal saline)	10ml/kg	96.00 ± 11.20	-
Cnestis ferruginea	300mg/kg	95.90 ± 7.20^{NS}	0.10
Cnestis ferruginea	400mg/kg	57.00± 3.70*	40.63
Cnestis ferruginea	500mg/kg	49.90 ± 3.43+	48.02
Indomethacin	10mg/kg	45.80 ± 3.60+	52.29

Each value is the mean ± SEM
**P ≤ 0.05 compared with control*
+P ≤ 0.001 compared with control
NS- not significant compared with control

Cotton pellet granuloma tissue formation test

The methanol extract of CF produced an inhibition of granuloma tissue formation which was significant at 400 ($p<0.05$) and 500mg/kg ($p<0.001$) dose levels.The highest inhibition ($p<0.001$) was produced by the reference drug (indomethacin, 10mg/kg) compared with the control (Table 5)

Discussion

The present study established the analgesic and anti-inflammatory activities of methanol extract of dried leaves of *Cnestis ferruginea*. Phytochemical analysis of plant extract revealed the presence of flavonoids, alkaloids saponins and anti-oxidants

the C-fibres and the second an inflammatory pain response. The extract also exhibited its analgesic activity by inhibiting the acetic acid -- induced writhing in mice [17]. This test is generally used as a model of visceral pain, however, the test is nonspecific as several other compounds such as tricyclic antidepressants [18] and antihistamines [19] also exhibit the writhing induced by acetic acid hence the need for other analgesic tests as we did in the present study. The hot plate test was next selected because the test is sensitive to strong analgesics and tissue damage is limited because of the cut off point that is usually imposed to limit the time spent on the hot plate.

level was not significant ($p>0.05$) in the early phase, the inhibition obtained in the late phase was significant ($p< 0.05$) compared with the vehicle treated group (control) (Table 1).

Hot plate test

The extract of CF, dose dependently produced a prolongation of the hot plate latencies which was significant at 500mg/kg dose level ($p<0.001$) at 60 min and at 400 and 500mg/kg dose levels ($p<0.001$) at 90 min.The reference drug (indomethacin,10mg/kg) also caused a prolongation of the hot plate latency significant at 30 and 60 min ($p<0.05$) and at 90mins ($p<0.001$) (Table 3).

Table 1: The effect of the methanol extract of Cnestis ferruginea on licking time in the formalin induced paw licking in rats

Groups	Oral dose	Early phase licking time (s)	Late phase licking time (s)
Control (normal saline)	10ml/kg	3.12 ± 0.35	7.07 ± 0.55
Cnestis ferruginea	300mg/kg	2.47 ± 0.32^{NS}	4.72 ± 4.72^{+}
Cnestis ferruginea	400mg/kg	2.13 ± 0.15*	4.03 ± 0.01^{+}
Cnestis ferruginea	500mg/kg	1.35 ± 0.17^{+}	2.99 ± 2.99^{+}
Indomethacin	10mg/kg	0.99 ± 0.15^{+}	2.52 ± 2.52^{+}

Each value is the mean ± SEM of six rats
**P ≤ 0.05 compared with control*
+P ≤ 0.001 compared with control
NS not significant compared with control

Acetic acid induced writhing in mice

The result showed that pre- treatment with the methanol extract of CF at all doses (300-500mg/kg) significantly ($p<0.001$) inhibited the writhing response in all the animals (Table 2).

Table 2: The effect of the methanolic extract of Cnestis ferruginea on acetic induced writhing in mice

Groups	Oral dose	Number of writhings	Percentage inhibition
Control (normal saline)	10 ml/kg	34.50 ± 4.20	-
Cnestis ferruginea	300mg/kg	31.50 ± 1.90^{+}	8.70
Cnestis ferruginea	400mg/kg	15.30 ± 1.20^{+}	55.60
Cnestis ferruginea	500mg/kg	14.50 ± 1.70^{+}	58.70
Indomethacine	10mg/kg	14.50 ± 0.90^{+}	58.00

Each value is the mean ± SEM of six mice
**P ≤ 0.05 compared with control*
+P ≤ 0.001 compared with control

Table 3: The effects of methanolic extract of Cnestis ferruginea on hot plate latencies in rats

		Reaction Times (Secs)			
Groups	**Oral dose**	**0 min**	**30min**	**60min**	**90min**
Control	10 ml/kg	11.58 ± 1.08	15.00 ± 1.51	15.33 ± 2.13	13.00 ± 1.67
Cnestis ferruginea	300mg/kg	15-00 ± 0.89	16.00 ± 0.63^{NS}	18.17 ± 1.22^{NS}	20.33 ± 1.43^{+}
Cnestis ferruginea	400mg/kg	14.67 ±1.74	21.40 ± 2.92^{NS}	23.67 ± 3.52^{NS}	24.50 ± 4.54^{+}
Cnestis ferruginea	500mg/kg	15.00 ± 1.03	21.00±2.07^{+}	23.00±0.68^{+}	23.17 ± 1.11^{+}
Indomethacin	10mg/kg	15.00± 1.51	20.00± 1.39*	22.00 ± 1.34*	25.00±1.88+

Each value is the mean ± SEM of six rats
**P ≤ 0.05 compared with control*
+P ≤ 0.001 compared with control
NS not significant compared with control

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The extract produced a dose dependent prolongation of the hot plate latency. This test is supraspinally mediated and is therefore a test of central activity, it therefore follows that the possibility of the extract having a central activity is very high.

The extract also has an anti-inflammatory activity as revealed by the carrageenan induced paw edema and cotton pellet granuloma tests. The extract significantly inhibited the carrageenan–induced paw edema formation. This test has a significant predictive value for anti-inflammatory agents acting by inhibiting mediators of inflammation [14].

Carrageenaan induced paw edema test is highly sensitive to non-steroidal anti-inflammatory agents and it has long been accepted as a useful phlogistic tool for investigating new anti-inflammatory drugs. The initial phase of the test is mediated by serotonin and histamine and mediators of the late phase are suspected to be arachidonate metabolites producing edema after mobilization of the neutrophils.

While the carrageenan-induced paw edema test is a test of acute inflammation, the cotton pellet granuloma test is a test of chronic inflammation [20]. The dry weight of the pellet has been shown to correlate with the amount of granuloma tissue formed [21]. At all doses, the plant extract significantly inhibited granuloma tissue formation dose dependently. Put together therefore, the two tests show that the plant extract can effectively reduce inflammation in both the acute and chronic phases.

In conclusion, the present study indicates that the methanol extract of *Cnestis ferruginea* possesses analgesic and anti-inflammatory properties thereby validating its local use by the alternative medical practitioners.

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des crises répétées (à cause d'offre limitée) était de 18,2%, ce qui n'était pas significativement différente de la mortalité de 20,0% enregistrée au cours des deux années (2008/2009), le médicament a été utilisé systématiquement comme l'unique agent anticonvulsif. P> 0,05. Les décès maternels étaient significativement plus observés parmi les femmes multipares avec l'éclampsie (23,3%) que le primigestes (16%), la mortalité était également plus accentuée chez les éclamptiques qui n'avaient pas de soins prénatals (18,7%) que ceux qui recevaient des soins (5,9%), p <0,05. Les complications graves maternelles étaient d'aspirations relatives à la pneumonie (23,9%) et d'œdème pulmonaire (16,3%), l'hyperthermie (17,9%), une insuffisance rénale aiguë (11,4%), et les accidents cérébro-vasculaires étaient de 9,8%. Le total des décès périnataux étaient de 392 avec 81,1% (318/392) encore des naissances et de 18,9% (74/392) des décès néonatals précoces essentiellement des asphyxies graves à la naissance. Le taux de mortalité périnatale était 406/1000.

Conclusion: L'incidence de l'éclampsie dans le groupe d'étude était élevée. C'était une cause majeure directe de la mortalité maternelle et périnatale. Le résultat de la maternité était également faible, même avec l'introduction de sulfate de magnésium. Les interventions pour la réduction de la mortalité maternelle et périnatale doivent mettre l'accent sur les stratégies qui empêchent l'apparition de l'éclampsie puisque les résultats dans certains contextes ne sont toujours pas encore favorables lorsqu'ils ne se produisent.

Introduction

Eclampsia is commonly defined as new onset of seizure activity during pregnancy or postpartum in a woman with signs or symptoms of preeclampsia [1]. Both pre-eclampsia and eclampsia account for significant maternal and fetal morbidity and mortality. Eclampsia is estimated to account for 50,000 maternal deaths worldwide annually [2] with 35% of patients having at least a major complication [3]. It is also associated with a fetal mortality rate of 12 % [4].

Eclampsia is a largely preventable condition but its prevention depends very much on the ability to detect early and appropriately manage pre-eclampsia in a pregnant woman. Poor utilisation of antenatal care services and late presentation to health care facilities contribute to the high number of cases of eclampsia in resource-poor countries. Hence, eclampsia continues to be one of the leading complications of pregnancy and also one of the leading causes of maternal and perinatal deaths.

However, the contribution of eclampsia to maternal and perinatal morbidity and mortality varies from one region of the world to another and from one country to another. For example, in Nigeria, eclampsia ranks amongst the first three major causes of maternal mortality [5,6,7]. The benefits of magnesium sulphate in the management of eclampsia had been established [8]. But it was only in 2008, that the use of magnesium sulphate became routine in the unit (Department of Obstetrics and Gynaecology, Usmanu Danfodiyo University Teaching Hospital, Sokoto) for all patients with eclampsia after the training of staff and regular supply of the drug courtesy of the Federal Ministry of Health were established. Also with eclampsia as a leading cause of maternal mortality in the institution [5], a High Dependency Unit (HDU) was established adjacent to the labour ward specifically for the care of patients with eclampsia and severe pre-eclampsia. Amongst other support facilities, the unit has multi-parameter monitors for patients, liberal supply of oxygen and dedicated nurse/midwives that are trained in life saving skill (LSS) including the administration of magnesium sulphate injection.

Post mortem examinations are not routinely performed in many centres like ours to ascertain the exact cause of death in eclampsia because of cultural and religious reasons. As a proxy, some measures like clinical audit meetings are conducted regularly. As a policy, the Department holds monthly maternal mortality reviews with representatives of other relevant units and departments, during which contributory factors to maternal deaths are discussed and consensus reached on the most likely cause of maternal death using clinical parameters and investigation results. Attendance at the meeting normally includes consultant obstetricians, trainees, midwives, paediatricians, anaesthetists, physicians and occasionally the hospital administrators.

Information and trends on fetal and maternal outcome may serve as a useful tool in developing other management strategies aimed at reducing maternal and perinatal mortality. This study was therefore undertaken to determine pregnancy outcome in women with eclampsia especially the maternal and perinatal deaths and the various contributory factors.

Methodology

The study was a retrospective analysis of demographic and clinical data of patients with eclampsia over a ten-year period with particular reference to fetal and maternal outcome.

The records of 1035 eclamptics admitted in O and G department of Usmanu Danfodiyo University