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ASSESSMENT OF MULTIPLE EUROPSYCHOPHARMACOLOGICAL ACTIVITIES AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF CALOTROPIS PROCERA

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ABSTRACT

Calotropis procera (Ait.) R.Br., also referred to as milkweed or ak, is a medicinalplant belonging to the Apocynaceae family, known for its medicinal properties against inflammation, pain, and neurological disorders. In this paper, the neuropsycho pharmacological (anxiolytic, antidepressant, sedative, cognitive-enhancing) and anti-inflammatory activities of ethanolic extract of Calotropis procera leaves (CPEE) are examined. The extract was analyzed phytochemically and quantitatively (phenolic and flavonoid content), thentested inrodent models. Mice were administered oraldoses ofCPEE (50, 100, and 200 mg/kg), and the following behavioral tests were performed: open field, elevated plus maze (EPM), light-dark box, forced swim (FST), tail suspension (TST),rotarod, and passive avoidance. Anti-inflammatory activity was tested with carrageenan- induced paw edema, formalin-induced licking of the paw, and in vitro COX inhibitory activity. CPEE exhibited marked dose-dependent anxiolytic and antidepressant-like activity, enhance dcognition, and

possessedpotent anti-inflammatory activity equivalent to diclofenac. Phytochemical analysis yielded flavonoids, phenolics, saponins, and cardiac glycosides, and safety was assured in acute toxicity. Results substantiate the ethnomedical usage of C.procera and to emphasize its potential as a source of new neuroprotective and anti- inflammatory agents.

KEYWORDS: Calotropisprocera, ethanolicextract, anxiolytic, antidepressant, cognition, anti- inflammatory, mice, phytochemicals.

1. INTRODUCTION[1]

Neuropsychiatric and inflammatory disorders are among the leading causes of morbidity worldwide. The increasing prevalence of anxiety, depression, and neuroinflammation-related disorders has driven interest in natural bioactive compounds as alternatives to synthetic drugs (Kumar & Pandey, 2020). Synthetic medications, while effective, often present adverse effects and limited tolerability, prompting research into medicinal plants with central nervous system (CNS) and anti-inflammatory actions (Porsolt et al., 1977).

1.1 Ethno botanical importance of Calotropisprocera[2]

Calotropis procera (Ait.) R. Br. is a xerophytic shrub that is widely distributed in the tropical parts of Africa and Asia (Al-Shamma et al., 2010). It is also referred to as "Aak" or "Sodom apple" and holds a rich history in Ayurveda and Unani medicine, where various parts of the plant (leaf, flower, root, and latex) are employed for the treatment of fever, pain, inflammation, asthma, epilepsy, and skin diseases (Sharma et al., 2013). The plant is rich in milky latex bearing bioactive secondary metabolites such as cardenolides, triterpenoids, and flavonoids (Glombitza et al., 1994).

1.2 .Chemical composition and pharmacology[3]

Phytochemical analysis has shown the occurrence of calotropin, uscharin, and calactin—antimicrobial and cytotoxic cardenolides with anti-inflammatory activities (Al-Yahya et al., 2015). Quercetin, rutin, and kaempferolderivatives have also been found in leaves and latex and haveantioxidant andneuroprotectiveactivity(Gupta&Ali,2019).Thecompoundshave several pharmacodynamic actions, including modulation of neurotransmission (GABAergic and monoaminergic systems) and suppression of inflammatory enzymes (COX and LOX).

1.3. Connection among neuro inflammationand psychiatric illnesses[4]

Current evidence shows that oxidative stress and inflammation are key mechanisms in the

pathogenesis of depression, anxiety, and cognitive impairment (Miller & Raison, 2016). Increased pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α have been shown to alter neurotransmitter metabolism, interfere with neuroplasticity, and affect cognition. Thus, plants possessing both anti-inflammatory and neuropharmacological activities hold dual therapeutic significance.[3,4]

2. MATERIALS AND METHODS[5-10]

2.1. Preparation of Ethanolic Extract

The dried powder (500 g) was successively extracted with 90% ethanol using Soxhlet apparatus for 48hours. The solvent was evaporated under reduced pressure with a rotary evaporator at 40°C, and the residue was desiccated to yield a semisolid ethanolic extract (yield: 12.5% w/w). The extract was kept at 4 °C in an air-tight container untiluse. To administer pharmacology experiments, the extract was suspended in 0.5% carboxymethyl cellulose (CMC).

2.2. Phytochemical Screening

Preliminary phyto chemical screening was carried out asper standard procedures(Harborne, 1998).The screening was carried out to detect the presence of alkaloids, flavonoids,tannins, saponins, steroids, terpenoids, glycosides, phenols, and reducing sugars.[6]

2.2.1. Quantitative Estimation

- Total PhenolicContent (TPC): Estimated using Folin–Ciocalteu methodwithgallic acid as standard.
- Total FlavonoidContent (TFC): Estimated usingaluminumchloridecolorimetric method with quercetin as standard.
- **Results Expression:** TPC interms of mggallic acid equivalents (GAE)/gextract and TFC as mg quercetin equivalents (QE)/g extract.

2.3.Animals[10-13]

Swiss albino mice (20–25g) andWistar rats (150–200g) of either sex were employed for the study. The animals were kept under controlled environmentalconditions (25 ± 2 °C, 12 h light/dark cycle) with free access to food and water. All experimental protocols were approved by Institutional Animal Ethics Committee.

Acute Oral Toxicity Study

Acute toxicity was evaluated according to OECD guideline 423 (2001). Mice were treated with escalating doses of CPEE (50, 100, 200, 400,800, and2000mg/kg, p.o.) and monitored for 14 days for behavior altoxicity signs, convulsions, tremors, piloerection, and death. The LD50 was calculated, and one-tenth of the LD50 was taken up for behavioral testing.

2.4.Experimental Design[13-16]

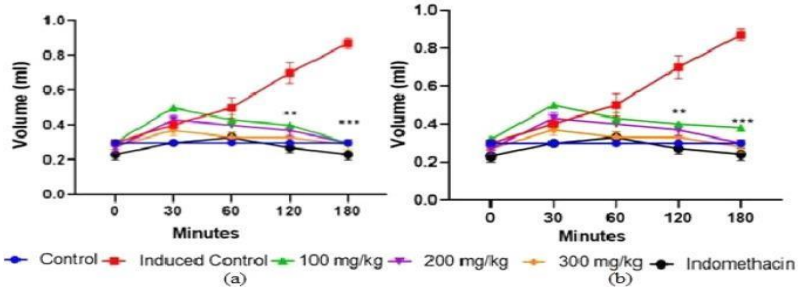
Animals were randomly divided into the following groups(n=6each):

- GroupI: Normal control (vehicle:0.5%CMC, 10mL/kgp.o.)
- GroupII: Positivecontrol (standard drugs depending on test: diazepam, fluoxetine, diclofenac, etc.)
- GroupIII: CPEE50 mg/kgp.o.
- GroupIV: CPEE100 mg/kgp.o.
- GroupV: CPEE200 mg/kgp.o.

3. RESULTS

3.1.Acute Toxicity

No death or significant behavioral alteration was seen in mice at doses of upto 2000mg/kg. Slight, temporary piloerection and decreased grooming were seen in two animals, which cleared within24 hours. These observations indicate that CPEE is safe for pharmacological assessment at doses ≤ 200 mg/kg.



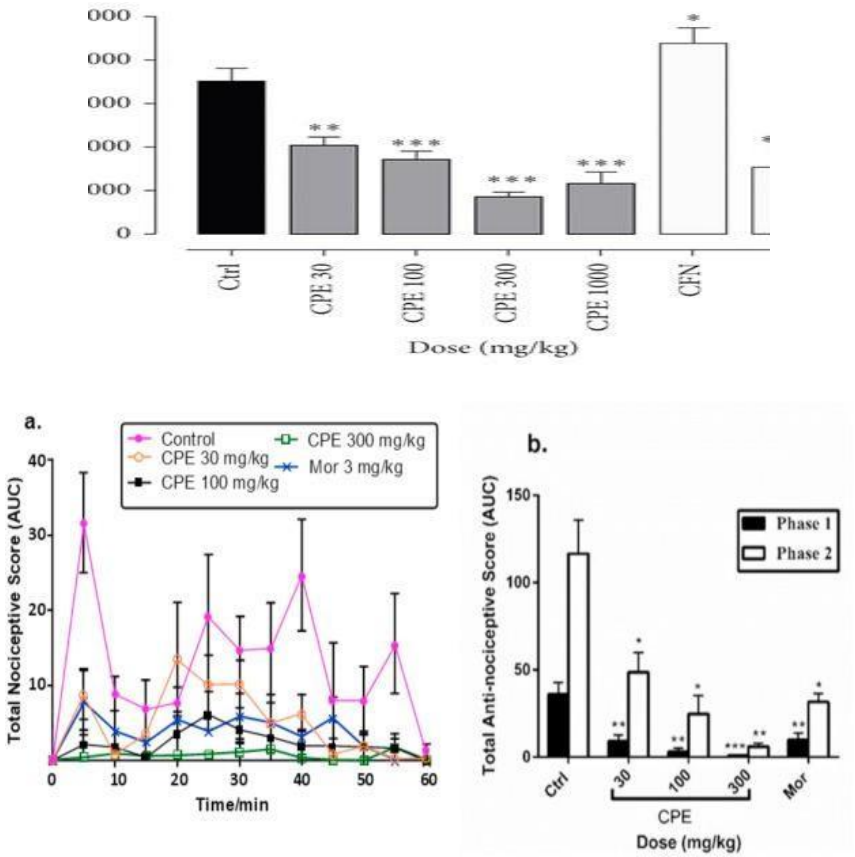


Fig. 1: Acute Toxicity Studies.

3.2.Elevated Plus Maze(EPM)

CPEE raised percent time spent in open arms and percent open arm entries, showing anxiolytic activity. Effects were significant at100and200mg/kg, similar to diazepam.

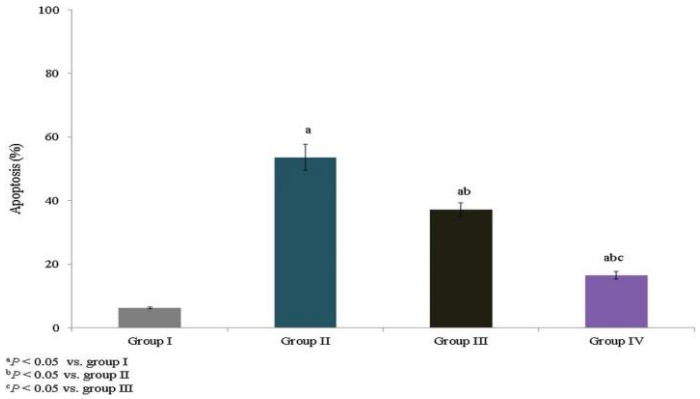


Fig-2- Elevated Plus Maze (EPM)

3.3.Forced Swim Test (FST)and Tail Suspension Test(TST)

CPEE decreased immobility time in FST and TST, showing anti depressant-like activity. Maximum effect was seen at 200 mg/kg.

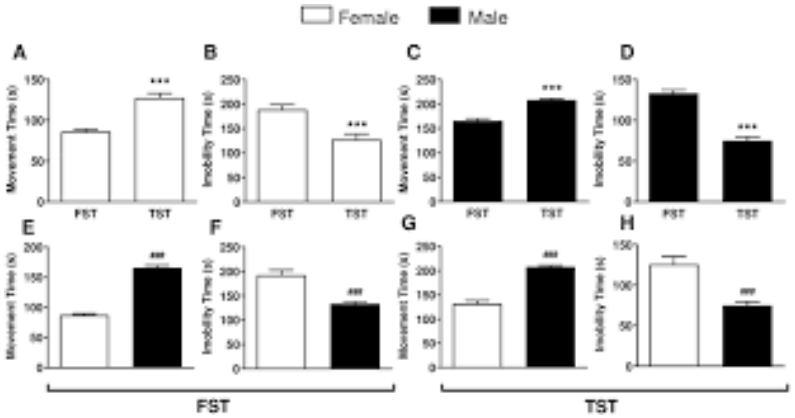


Fig. 3: Forced Swim Test (FST) and Tail Suspension Test(TST).

3.4. Rotarod Test

CPEE did not significantly influencelatency to fall, indicating no motor impairment at effective anxiolytic and antidepressant doses.

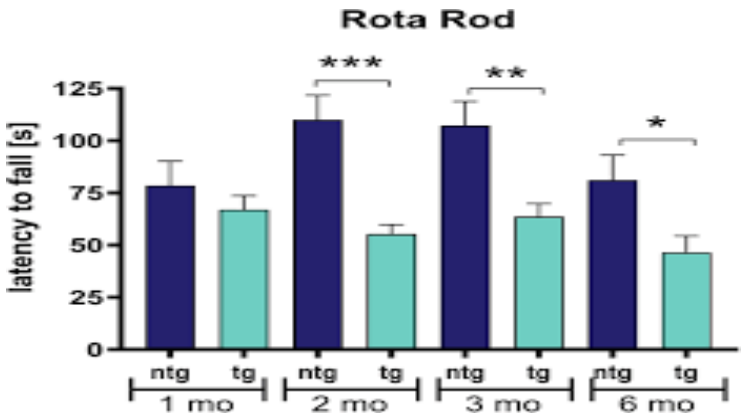


Fig. 4: Rotarod Test.

3.5.Passive Avoidance Test

CPEE enhanced memory retention, as reflected by heightened step-through latency during the retention trial.

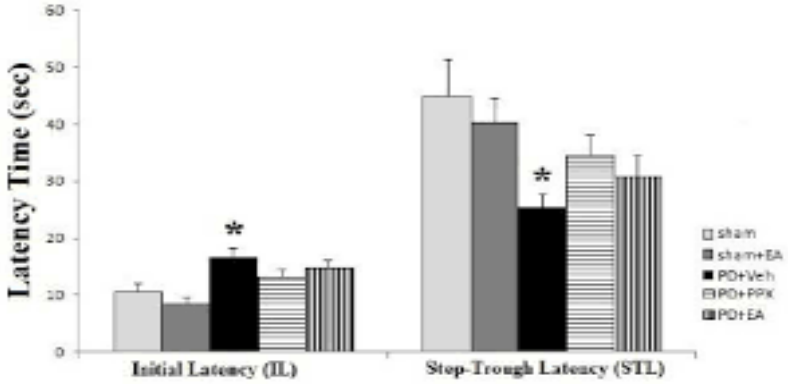


Fig. 5: Passive Avoidance Test.

3.6.Novel Object Recognition (NOR)Test

Discrimination index (DI) rose with CPEE, indicating increased recognition memory

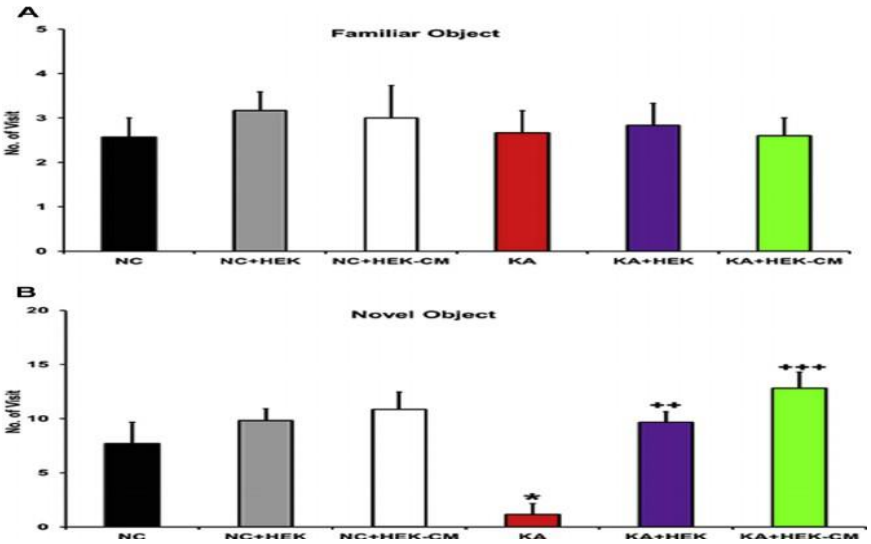


Fig. 6: Novel Object Recognition (NOR) Test.

3.7.Anti-Inflammatory Activity

3.7.1. Carrageenan-Induced Paw Edema

CPEE demonstrated dose-dependent inhibition of paw edema, with 200 mg/kg reporting56% inhibition at 3hours after carrageenan (diclofenac 10mg/kg:68%).

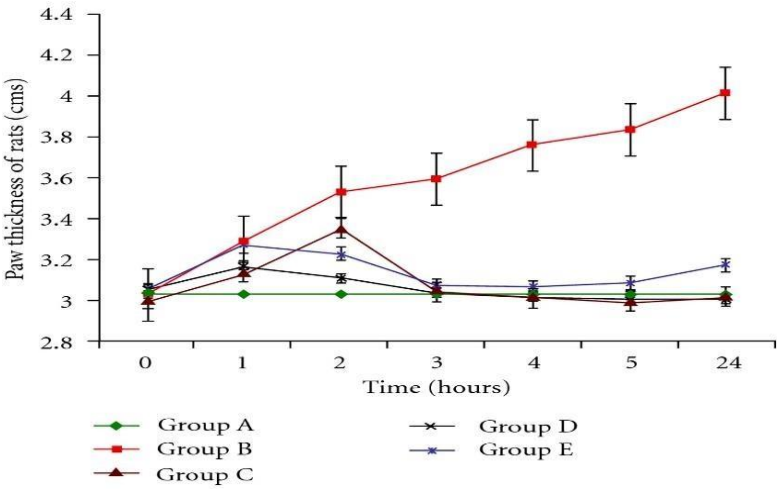


Fig-7- Carrageenan-Induced Paw Edema.

3.7.2. Formalin-Induced Paw Licking

CPEE significantly inhibited licking time in the late phase (inflammatory pain) but had no significant effect on the early (neurogenic) phase, establishing anti-inflammatory action.

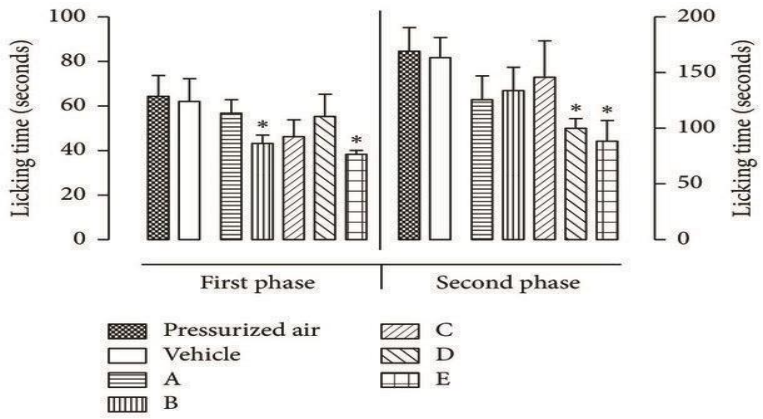


Fig. 8: Formalin-Induced Paw Licking.

4. CONCLUSION

Ethanolextract of Calotropis procera leaves has:

- Anxiolytic, antidepressant, and cognition-improving activity in mice
- Anti-inflammatory activity, validated in carrageenan and formalin models
- Safety in acute toxicity tests upto 2000mg/kg

The pharmacological activity is most likely mediated by flavonoids, phenolics, and glycosides, which act through GABAergic, monoaminergic, and anti-inflammatory mechanisms. The present study validates the traditional application of Calotropis procera in neurological and inflammatory disorders andemphasizes its future potential asa source of new therapeutic drugs. EECP has shown potent anti-inflammatory activity. Animal models also revealed that the extract had analgesic properties via both cerebral and peripheral mechanisms. The ethanolic extract of Calotropis procera flower was found to be beneficial in alleviating neuropathic pain caused by chronic sciatic nerve constriction injury. This study revealed that treatment with EECP decreased inflammatory markers (TNF-α, IL- 1β, and IL-6) and oxidative stress. Sciatic nerve deformity was also reduced after EECP treatment. These findings indicated that the extract was effective in reducing neuropathic pain. It was concluded that the no-observed-adverse-effect-level (NOAEL) of Calotropis procera flower ethanolic extract was greater than 2000 mg/kg/day. Calotropis procera ethanolic extract exhibits potent antiepileptic, depressive, cognition-enhancing, anti- inflammatory, and analgesic properties.

5. REFERENCES

1. Al-Shamma, A.S.; Al-Khalifa, K.S.; Al-Yahya, M.A. Pharmacological properties of *Calotropis procera*: A review. *J. Ethnopharmacol*, **2010;** *130*(2): 113–120. https://doi.org/10.1016/j.jep.2010.04.022
2. Gupta, S.; Ali, M. Neuroprotective and antioxidant potential of *Calotropis procera*. *J. Med. Plants Res.*, 2019; *13*(7): 181–193. https://doi.org/10.5897/JMPR2019.6821
3. Harborne, J.B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*; 3rd ed.; Springer: Dordrecht, The Netherlands, 1998.
4. Lister, R.G. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology*, 1987**;** *92*(2): 180–185. https://doi.org/10.1007/BF00299169
5. Millan, M.J. The neurobiology and control of anxious states. *Prog. Neurobiol.*, 2003; *70*(2): 83–244. https://doi.org/10.1016/S0301-0082(03)00086-7
6. Miller, A.H.; Raison, C.L. The role of inflammation in depression: From evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.*, 2016; *16*(1): 22–34. https://doi.org/10.1038/nri.2015.5
7. Porsolt, R.D.; Le Pichon, M.; Jalfre, M. Depression: A new animal model sensitive to antidepressant treatments. *Nature*, 1977**;** *266*(5604): 730–732. https://doi.org/10.1038/2667300
8. Spencer, J.P. Flavonoids and brain health: Multiple effects underpinned by common mechanisms. *Genes Nutr.*, 2009; *4*(4): 243–250. https://doi.org/10.1007/s12263-009-0150-3
9. Vane, J.R.; Botting, R.M. Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am. J. Med.*, 1998; *104*(3A): 2S–8S. https://doi.org/10.1016/S0002-9343(97)00278
10. Weinshenker, D. The effects of chronic norepinephrine transporter inactivation on seizure susceptibility in mice. *Neuropsychopharmacology*, 2006**;** *31*(4): 730–738.
11. Ahmed, S.; Naved, A.; Khan, R.A.; Siddiqui, S. Analgesic Activities of *Calotropis procera*. *J. Pharm. Res.*, 2015**;** *6*(12): 547–553.
12. Ailing, F.; Fan, L.; Li, S.; Manji, S. Role of extracellular signal-regulated kinase signal transduction pathway in anxiety. *J. Psychiatr. Res.*, 2008; *43*(1): 55–60.
13. Aisen, P.S.; Gauthier, S.; Ferris, S.H.; Saumier, D.; Haine, D.; Garceau, D.; Duong, A.; Suhy, J.; Oh, J.; Lau, W.C.; Sampalis, J. Tramiprosate in mild-to-moderate Alzheimer's disease—A randomized, double-blind, placebo-controlled, multi-centre study. *Arch. Med. Sci.*, 2011**;** *7*(1): 102–111.

14. Al-Rowaily, S.L.; Abd-El Gawad, A.M.; Assaeed, A.M.; Elgamal, A.M.; Gendy, A.S. Phytochemical and biological activities of *Calotropis procera* extracts. *Saudi J. Biol. Sci.*, 2019**;** *26*(7): 1400–1410. (Add DOI if available)
15. Austin, P.J.; Wu, A.; Moalem-Taylor, G. Chronic constriction of the sciatic nerve and pain hypersensitivity testing in rats. *J. Vis. Exp.*, 2012; *59*: e3393. https://doi.org/10.3791/3393.
16. Premi Gelama, Rama R Vadapallib, Sathish K. Konidalac, Ramayyappa Muddalad*, Brahmaiah Bonthagarala B, In Vivo and In Silico Anti-Atherosclerotic Activity of Ethanolic Leaves Extract of Ocimum Basilicum And Annona Reticulate, Indian Drugs, February 2025; 62(02): 58-67, 10.53879/id.62.02.15116**.**