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Effect of *Prosopis juliflora* Alkaloids on Brain and Cardiovascular System

A. KANTHASAMY[a], K. GOPALARATHINAM[b], S. GOVINDASAMY[a]

[a] Department of Biochemistry, University of Madras, Guindy Campus, Madras - 600 025, India
[b] Department of Physiology, V.H.S. Medical Centre, Madras - 600 113, India

PROSOPIS JULIFLORA ALKALOİDLERİNİN BEYİN VE KALP-DAMAR SİSTEMİ ÜZERİNE ETKİLERİ

Özet

Prosopis juliflora' dan saflaştırılan alkaloidlerin, sıçan beyin ve kalp-damar sistemi üzerindeki toksik etkisi araştırıldı. EEG, EKG ve kan akışı kayıtları yapıldı, ayrıca, akut doz yüklemeleri sonrasında beyin asetilkolinesteraz, asetilkolin ve Na^+, K^+ -ATPaz düzeyleri incelendi. EEG bulguları ile asetilkolinesteraz, asetilkolin, Na^+, K^+ -ATPaz düzeylerindeki değişiklikler, alkaloidlerin antikolinerjik etkilerine bağlandı. EKG ve kan akışı incelemelerinde, alkaloidlerin neden olduğu ve değişik zaman aralıklarında ortaya çıkan kardiovasküler düzensizliklere ait değişiklikler tesbit edildi. Atropinin, *Prosopis juliflora* alkaloidleri üzerindeki antagonist etkisi, bu alkaloidlerin kalp-damar sistemi üzerindeki etkilerini kolinerjik bir mekanizmayla gösterdiği şeklinde yorumlandı. Bu bulgulara dayanarak, alkaloidlerin toksik etkilerini kolinerjik inhibisyonu izleyen kalp-damar sisteminde durma şeklinde gösterdiği varsayıldı.

Summary

To evaluate the toxic influence of *Prosopis juliflora* alkaloids, the alkaloids were isolated and tested on rat brain and cardiovascular systems. EEG, ECG and blood flow were recorded and also the levels of brain acetylcholinesterase, acetylcholine, and Na^+, K^+ - ATPase were observed in acute dose administration. Alterations in EEG patterns and acetylcholinesterase, acetylcholine, Na^+, K^+ - ATPase levels revealed the anticholinergic action of the alkaloids. The changes in ECG and blood flow in different time intervals showed the cardiovascular abnormalities induced by the alkaloids. The antagonistic effect of atropine to *Prosopis juliflora* alkaloids suggest that the alkaloids effect on cardiovascular system are cholinergic in nature. Hence, the toxic influence of the alkaloids are mainly through cholinergic inhibition followed by cardiovascular arrest.

Keywords: *Prosopis juliflora - Alkaloids - Cholinergic inhibition*

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INTRODUCTION

Prosopis juliflora A.DC., belonging to the family *Leguminosae*, is a poisonous plant which is widely distributed all over the world (1). The crushed leaves of the plant have been used as suicidal and homicidal agents in rural parts of Southern India. In addition, the bark of *P. juliflora* has been used for the preparation of illicit arrack in rural areas. Hence, the plant has forensic importance and proper exploration of the biochemical action of the active principles *P. juliflora* in biological system may be of immense help to forensic toxicologists, biochemists and pharmacists. Eventhough the chemical data about different active principles of *P. juliflora* such as glycosides (2), flavonoids (3), tannins (4) and highly complex indolizidine alkaloids (5,6) have been reported, details regarding the biochemical alterations and the mode of action are enigmatic. Recently, we have reported the hemolytic effect of *P. juliflora* alkaloids in rat and human erythrocytes (7). In the present work, an earnest attempt has been made to unravel the mode of action and biochemical alterations of *P. juliflora* alkaloids on vital systems like nervous and circulatory systems by investigating classical electrophysiological studies like EEG, ECG and blood flow which are effectively supported by biochemical parameters.

MATERIALS AND METHODS

P. juliflora leaves were collected, dried and pulverized. The pulverized leaves are subjected to hexane followed by benzene extractions to remove lipid and chlorophyll materials. The defatted leaf powder was subjected to repeated cold methanol extraction. The dark brown alkaloidal fraction was obtained from the methanol extract by following the method of *Oot-Longoni* et *al* (8).

Male Wistar rats weighing 130-150 g were used in the present investigation. They were maintained in food and water *ad libitum*. An acute dose of *P. juliflora* alkaloids, 25 mg/kg body weight dissolved in 0.5% of citric acid was administered intraperitoneally. EEG was recorded in an eight channel EEG machine (MEDICARE) from FP2-02 region of brain and EEG from one channel was split up into delta, theta, alpha and beta bands by four filters. ECG and blood flow were recorded separately in the classical polyride instrument by using limb leads and photo optic pleathesmograph. The brain was removed immediately after death of the experimental animals and homogenized in 0.1M Tris-HCI buffer at 4°C. The homogenate was used for the estimation of acetylcholinesterase (AchE), Na^+, K^+ dependent ATPase and acetylcholine (Ach) content. The control animals receiving 0.5% citric acid were also sacrificed along with experimental rats and the above biochemical parameters were carried out. AchE and Ach content were estimated by the methods of *Ellman* et *al* (9) and *Hesterin* (10), respectively. *Bonting's* method (11) was adopted for the estimation of Na^+, K^+ dependent ATPase.

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RESULTS AND DISCUSSION

The EEG recordings from acute dose in rats for *Prosopis juliflora* alkaloids is presented in Figure 1. The 0-time recording was considered as normal EEG signals which was recorded before alkaloids administration. After 6-7 min of the alkaloids administration, violent convulsions started and abnormal EEG wave patterns of bursts mixed with multiple spikes were recorded both in main channel as well as in split up waves delta, theta, alpha and beta. Just before death (about 2 min) feeble brain electrical activity was noted. Around 18 min after the alkaloid injection, the animal was found dead and no electrical discharges were recorded in EEG. Similar to our observation of polyspike with high voltage discharges followed by decreased electrical potentials were observed during convulsent administration (12). Hence, our results reveal the fact that *Prosopis juliflora* alkaloids might be convulsent in nature. These findings were further strengthened by biochemical studies on brain parameters namely AchE, Ach content and Na^+, K^+ -ATPase which are presented in Table I.

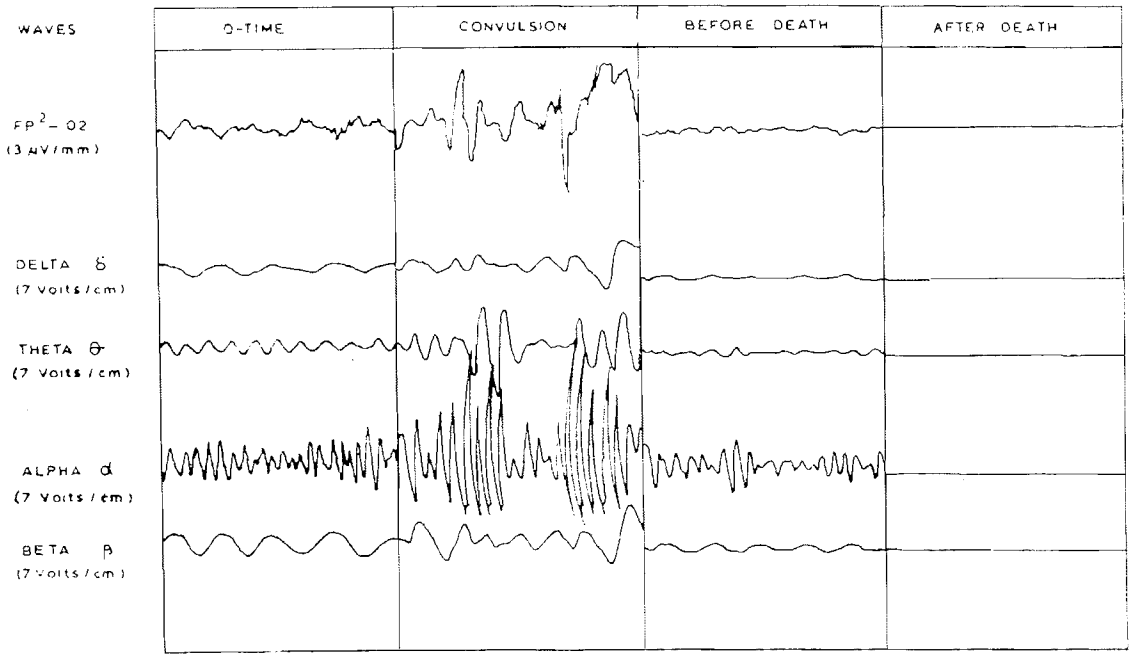

Figure 1. Effects of *Prosopis juliflora* alkaloids on EEG in experimental rat.

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Table I. Levels of acetylcholinesterase (mU/mg protein), acetylcholine (nM/g wet tissue) and Na^+, K^+ -ATPase (m mc mole/mg protein/h r) in control and *Prosopis juliflora* alkaloids administered rats (test).

	Control	Test
Acetylcholinesterase	937.24 ±48.23	483.96 ± 15.49*
Acetylcholine	18.80 ± 1.26	27.53 ± 1.37*
Na^+, K^+ -ATPase	2382 ± 102	1496 ± 94 *

* $p < 0.001$.
The values are expressed as mean ± SD from six individual experiments.

Decreased activity of AchE and a concommitent increase in Ach was noted in experimental rats when compared with control animals. Decreased activity of Na^+, K^+ -ATPase was also observed in experimental rats. Inhibition of AchE and accumulation of Ach in convulsion inducing agents have bleen reported (13).

It has been suggested that Ach and Na^+, K^+ -ATPase system of brain have a common link (14) and *Parton* et *al* (15) presented evidence that conditions known to inhibit Na^+, K^+ -ATPase were able to alter the release of Ach from nerve terminals. Further, Na^+, K^+ -ATPase plays a vital role in the nerve electrical potential by maintaining the balance of Na^+ and K^+ ions between nerve membranc (16). Hence, the decreased activity of Na^+, K^+ -ATPase in *Prosopis juliflora* alkaloids treated rats may alter the electrolyte balance in nerve fibre and thereby alter the electrical impulses of brain which is evident from the EEG observations. The above overall studies confirm the anticholinergic action of *P. juliflora* alkaloids.

The ECG and blood flow recording patterns of *Prosopis juliflora* alkaloids administered rats at different intervals of time is presented in Figure 2. The 0-time recording of ECG revealed the normal functioning of heart (420 beats/min) and blood flow showed the normal circulation. It is interesting to note the elevated peripheral blood flow without increase in the heart rate at 3rd min of recording. This finding led us to speculate that the effect of *P. juliflora* alkaloids on cardiovascular system is secondary to cholinergic inhibition. The accumulation of Ach causes vasodilation in peripheral blood flow (17). Further increase in the accumulation of Ach leads to convulsions around 7th min which lasted about 5 min and abnormal ECG and blood flow pattern were noted due to high electric discharges. After violent convulsions, the heart rate and blood flow were found to be decreased at 12th min which resulted in bradycardia and

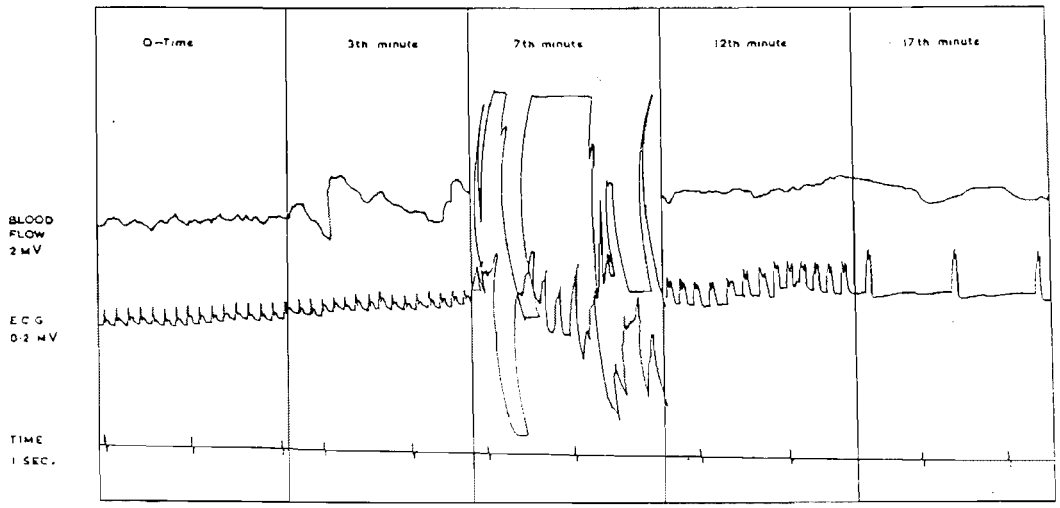

Figure 2. Effects of Prosopis juliflora alkaloids on ECG and blood flow in experimental rats.

finally death. Induction of circulatory collapse in addition to altered EEG by high concentration of Ach have also been reported (18).

The alterations in ECG and blood flow produced by *Prosopis juliflora* alkaloids was completely abolished by intravenous administration of the antimuscarinic drug atropine (1.0 mg/kg) 20 min before administration of the alkaloids. This antagonism by atropine suggests a cholinergic involvement in cardiovascular effects of the alkaloids.

From our observations it may be concluded that *Prosopis juliflora* alkaloids exert their toxic influence primarily through cholinergic inhibition accompanied by circulatory collapse.

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Reprinst request to :
Dr.A. Kanthasamy
Department of Biochemistry,
University of Madras, Guindy Campus,
Madras 600 025, India