



INTERACTIONS OF ALCOHOLIC EXTRACT OF *CYAMOPSIS TETRAGONOLOBA* L. FRUITS WITH ALCOHOL IN RABBITS

ABSTRACT

Cyamopsis tetragonoloba is a plant in the family Fabaceae. *Cyamopsis tetragonoloba* (L) Taub is a moderate sized annual herb found throughout India as a cultivar for its pods used as vegetable. Plant is popular in indigenous system of medicines like Ayurveda, Siddha and discussed in various traditional literatures. In traditional medicines various parts such as leaves, seeds and pods are used in diabetes, asthma, inflammation, as Laxative, antibilious, appetite depressor (weight loss not observed) and hypolipidemic agent. The fruits of *Cyamopsis tetragonoloba* L. were successively extracted with n-hexane and Alcohol by soxhlet extraction. The alcoholic extract was dried and preliminary phytochemical screening was done, which revealed the presence of alkaloid, saponin, flavanoids and cardiac glycosides. The alcoholic extract was given to rabbits in 200 mg/kg and 400 mg/kg with Disulfiram as standard. Both the dose levels of extracts did not exhibited significant inhibition of Aldehyde dehydrogenase enzyme there by the elevated ketone bodies in rabbit serum was observed. The inhibition of alcohol dehydrogenase by the extract is not significant for treatment of alcoholism when compared to standard Disulfiram.

Key words: *Cyamopsis tetragonoloba*, Disulfiram, Aldehyde dehydrogenase, ketone bodies

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INTRODUCTION

The fruits of *Cyamopsis tetragonoloba* were used as anti-inflammatory agent, anti-oxidant, laxative. As per Ayurveda the plant is used to reduce fire and can be used as cooling, digestive, tonic, galactagogue, useful in constipation, dyspepsia, anorexia, Galatia, hyetalopia and vitiated condition of kapha and pitta. The leaves of the plant have been reported to have anti-asthmatic activity and to cure night blindness. ^[1] Majority of the people were habituated to Alcohol which leads to its addiction. Alcohol is the single most significant cause of liver disease throughout the world which accounts 60-80 % of cases of cirrhosis in various countries. Several studies revealed that 20% of lifelong alcoholics will develop significant liver disease.

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An estimated 20% of heavy drinkers develop progressive liver fibrosis, which leads to alcohol cirrhosis, typically after a period of 10-20 years of heavy indulgence. ^[2] Dependence on alcohol consumption becomes apparent 6-12 hours after cessation of heavy drinking as a withdrawal syndrome that may include tremor, nausea, vomiting, excessive sweating, agitation and anxiety. Generalized seizures may manifest after 24-48 hours of cessation, an alcohol withdrawal delirium may become apparent in which the person hallucinates, is disoriented, and shows evidence of autonomic instability. Delirium tremors are associated with 5-15% mortality. ^[3] Disulfiram which is an Aldehyde dehydrogenase inhibitor is commonly used drug in the treatment of Alcoholism. When alcohol is ingested after taking Disulfiram, the concentration of acetaldehyde in tissues and blood rises leading to Aldehyde syndrome. This includes- flushing, burning sensation, throbbing, headache, perspiration, uneasiness, tightness in chest, dizziness, vomiting, visual disturbances, mental confusion, postural fainting and circulatory collapse. Duration of syndrome depends on the amount of alcohol consumed. Because of risk of severe

reaction, Disulfiram is to be used with great caution, only in well-motivated subjects.^[4] The main objective of the present study is to De-addict the individuals suffering from Alcohol addiction and one of the approaches for De-addiction of Alcohol is by giving out the ethyl acetate extract of *Cyamopsis tetragonoloba* fruits.

MATERIALS AND METHODS

The fresh fruits of the plant *Cyamopsis tetragonoloba* were collected from Nandyal, Kurnool district; Andhra Pradesh, India in the month of August-September (flowering season) and the plant was identified and authenticated by from the botany department P. Prasad Rao, M.Sc., Dept. of Botany, PSC & KVSC Government Degree College and Nandyal.

Alcoholic Extract

Shade dried *Cyamopsis tetragonoloba* fruits were pulverized and the powder was initially defatted with n-hexane (60-80°C). The marc was further extracted with Alcohol in a soxhlet apparatus. The solvent of the extract was removed; the residue free from solvent is used for the studies. The extracted value was found to be 23.33% w/w.^[5]

EXPERIMENTAL WORK:

Experimental animals

Rabbits weighing about 1-1.5 kg were used for the study. Animals were kept in animal house at an ambient temperature of 25 C at 44-55% relative humidity, animals were fed with vegetables and water .CPCSEA guidelines for laboratory animal facilities were followed. Two animals are used for each step, the dose level to be used as the starting dose is selected from one of four fixed levels 50, 300,500, and 2000mg/kg body weight .The starting dose level should be that mortality is un likely at the highest starting dose level (2000mg/kg body weight) the time interval between treatment groups is determined by the onset, duration and severity of toxic signs. Treatment of animals at the next dose should be delayed until one is confident of survival of the previously dosed animals.

Experimental Protocol

Rabbits were randomly divided into five groups, each consisting of six animals.

Group1: Animals were administered with DMSO and served as normal.

Group2: Animals were administered with oral alcohol (2% of blood volume w/v of rabbit) served as control

Group 3: Animals were treated with oral alcohol (2% of blood volume w/v) and Disulfiram (100mg/kg, i.p.) served as standard

Group 4: Animals were treated with oral alcohol (2% of blood volume) and EAESGL (200mg/Kg, p.o) served as low dose

Group 5: Test group animals were treated with oral alcohol (2% of blood volume) and EAESGL (400mg/Kg, p.o) served as high dose

Note: DMSO was used as a solvent both for the extract and for the Disulfiram.

The rabbits were treated as per the treatment protocol and their body weights were monitored sequentially in control and experimental animals for period three hours.

Preparation of 2, 4-DNP^[6]

Dissolve 1.5 g of 2, 4-dinitrophenylhydrazine in 20 ml of sulphuric acid (50 percent v/v). Dilute to 100 ml with water and filter.

Estimation of ketone bodies^[7]

Blood was collected from retro orbital region of rabbit's eye once in an hour. Serum was separated by centrifuging at 5000 for a period 15 minutes at the temperature of 25°C. Serum was separated and treated with 2, 4-dinitro phenyl hydrazine (2, 4-DNP) reagent to develop a colour. The absorbance was measured against a blank using UV-Visible Spectrophotometer at 540nm.

RESULTS:

Acute toxicity study:

The EECT was found to be safe at the maximum single dose of 4000mg/kg when administered orally and the animals did not show any gross behavior changes hence according to OECD guidelines 420, 423 the dose can be reduced up to 1/10 of the dose that is 400mg/kg were used as high dose and 1/20 of the dose that is 200mg/kg were used as low dose in the subsequent study respectively.^[8] The study was approved and conducted as per the norms of Institutional Animal Ethics Committee (IAEC) of Santhiram college of Pharmacy (1519/PO/a/11/CPCSEA). The EECT was not as significant as Disulfiram in inhibiting the enzyme aldehyde dehydrogenase for treatment of Alcoholism. The duration of inhibition of alcohol dehydrogenase by the extracts is slower than the standard Disulfiram and lasted for 4 hrs but to lesser extent than the Disulfiram and the results was shown in Table 1 and fig no 1.

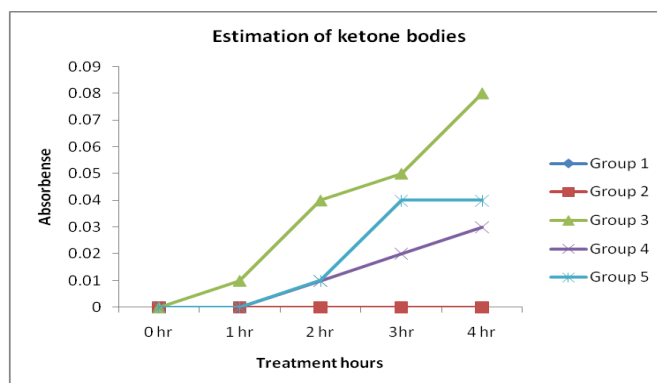


TABLE 1: Estimation of Ketone Bodies using Alcoholic extract of *Cyamopsis tetragonoloba* fruits

S. No	Group	Absorbance				
		0 hr	1 hr	2 hr	3hr	4 hr
1	Group 1	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
2	Group 2	0.00±0.0	0.00±0.0 ^{###}	0.00±0.0 ^{###}	0.00±0.0 ^{###}	0.00±0.0 ^{###}
3	Group 3	0.00±0.0	0.01±0.003 [*]	0.04±0.003 ^{**}	0.05±0.002 ^{***}	0.08±0.003 ^{***}
4	Group 4	0.00±0.0	0.00±0.000	0.01±0.002	0.02±0.003 [*]	0.03±0.003 ^{**}
5	Group 5	0.00±0.0	0.00±0.000	0.01±0.003 [*]	0.04±0.002 ^{**}	0.04±0.002 ^{***}

Values are Mean± S.E.M of 6 animals in each group. One way ANOVA used.*= P<0.05; **= P<0.01;***=P<0.001 Compared to Group-I

Statistical analysis:

Statistical analysis was carried by Graph Pad prism 5 statistical software. Results were analyzed by one way ANOVA (Analysis of variance) and the significance level was calculated using Tukey Kramer multiple comparison test.

DISCUSSION:

The Present study reports the interaction of Ethanolic extract of *Cyamopsis tetragonoloba* fruits on alcohol metabolism. Alcohol is metabolized by the enzyme Alcohol dehydrogenase and is converted to aldehyde in the liver by cytochrome P₄₅₀ enzyme systems. The Aldehyde thus formed is converted to Acetic acid by the enzyme Aldehyde dehydrogenase. Disulfiram is the drug which was widely available for De-addiction of alcohol which mainly acts by inhibiting the Aldehyde dehydrogenase enzyme. The results reveals that the Ethyl acetate extract of *Cyamopsis tetragonoloba* at the dose levels of 200mg/kg and 400mg/kg do not exhibit significant inhibitor of aldehyde dehydrogenase enzyme. Hence it is concluded that *Cyamopsis tetragonoloba* is not as significant as Disulfiram in treatment of Alcoholism.

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