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"INTERACTION OF ETHYL ACETATE EXTRACT OF *SESBANIA GRANDIFLORA LINN*. LEAVES ON ALCOHOL METABOLISM IN RABBIT''

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ABSTRACT

Sesbania grandiflora, a plant in family Leguminosae is found widely in Thailand. The leaf and flower of the plants has been utilized as food whereas its stem has been used as a traditional medicine. *Sesbania grandiflora*. Linn Leaves were broadly used in treatment of several diseases like CNS depressant, analgesic [1], anti-oxidant [2], microbial infections [3]etc. However, there is very few scientific data on biological activity of this plant. In the present study, the leaves of *Sesbania grandiflora* L. were successively extracted with n-hexane and Ethyl acetate (EA) by soxhlet extraction. The EA extract was dried and preliminary phytochemical screening was done, which revealed the presence of alkaloid, tannins, flavanoids, steroids and glycosides. Acute toxicity study was carried out using the fixed dose method according to OECD guideline no. 423. The different doses like 500, 1000, 2000, 3000 and 4000 mg/Kg body weight were administered orally to the animals, observed for 24 hr after dosing and also observed for 14 days without giving drug. EA extract was given to rabbits in 200 mg/kg and 400 mg/kg with Disulfiram as standard. Both the dose levels of extracts exhibited significant inhibition of Aldehyde dehydrogenase enzyme there by the elevated ketone bodies in rabbit serum was observed. The inhibition of aldehyde dehydrogenase by the extract was found to be less potent than the standard Disulfiram.

KEY WORDS: *Sesbania grandiflora*, Disulfiram, Aldehyde dehydrogenase, ketone bodies

INTRODUCTION

The leaves of *Sesbania grandiflora* were used as tonic, diuretic, laxative, antipyretic and chewed to disinfect mouth and throat. [4] 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. 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. [5] 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 withdrawl 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.[6] 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 *Sesbania grandiflora* leaves.

MATERIALS AND METHODS

COLLECTION OF *SESBANIA GRANDIFLORA* LEAVES

The fresh leaves of the plant *Sesbania grandiflora* were collected from panyam village, Kurnool district; Andhra Pradesh, India in the month of August (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, Nandyal.

DRUGS AND CHEMICALS

1. n-Hexane : MERCK specialties private limited
2. Ethyl Acetate : SDFCL SD fine-chem limited
3. DMSO: SDFCL SD fine-chem limited
4. Disulfiram
5. Alcohol: Santhiram college of Pharmacy

PREPARATION OF ETHYL ACETATE EXTRACT OF *SESBANIA GRANDIFLORA* LEAVES

Shade dried Sesbania leaves was pulverized and the powder was initially defatted with petroleum ether (60-80°C). The marc was further extracted with Ethyl acetate in a soxhlet apparatus. The solvent of the extract was removed and the residue free from solvent is used for the studies. The extracted value was found to be 27.77%w/w. [7]

EXPERIMENTAL ANIMALS

Either sex Wister albino rabbits weighing about 1.3 - 1.8 kg were used in the study. Animals were obtained from Sainath enterprises, Hyderabad. The study protocol was reviewed and approved by the institutional animal ethical committee of Santhiram College of pharmacy (1519/PO/a/11/CPCSEA). They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (approximately 12 h light/ 12 h dark) and maintained humidity 44-55% and an ambient temperature of 25±2%. All the animals were acclimatized 1 week before use. All experiments were performed between 9:00 am to 4:00 pm.

ACUTE TOXICITY STUDY

The acute oral toxicity study was carried out for ethyl acetate extract of *Sesbania grandiflora* leaves using the fixed dose method according to OECD guideline no. 423. Healthy adult albino rabbits weighing between 1.5 to 1.8 kg were used for the study. Animals were divided into six groups of six animals each and kept fasted overnight. The different doses like 500, 1000, 2000, 3000 and 4000 body weight were administered to the group I, II, III, IV, V respectively. After administering the ethyl acetate extract of *Sesbania grandiflora* leaves in different groups the behavioral changes, Eyes, Salivation, Diarrhea, Mortality etc. were observed for 24 hr (OECD guidelines) and also observed for 14 days without giving drug (OECD, 423).[8]

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

Group4: Animals were treated with oral alcohol (2% of blood volume) and EAESGL (200mg/Kg, p.o) served as lowdose

Group5: Test group animals were treated with oral alcohol (2% of blood volume) and EAESGL (400mg/Kg, p.o) served as highdose

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

0.02 g of 2, 4-DNP was weighed accurately and prepared freshly by dissolving in 100ml of 2N hydrochloric acid. [9]

ESTIMATION OF KETONE BODIES

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 25^0C then collected serum was treated with 2, 4-dinitro phenyl hydrazine (2, 4-DNP) regent to develop a colour. The absorbance was measured against a blank using UV-Visible Spectrophotometer at 540nm.

RESULTS

ACUTE TOXICITY STUDY

The EAESGL 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.[10] The Ethyl acetate extract exhibited Disulfiram like activity at both the tested doses. 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: 1Estimation of ketone bodies using ethyl acetate extract of *Sesbania grandiflora* leaves

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

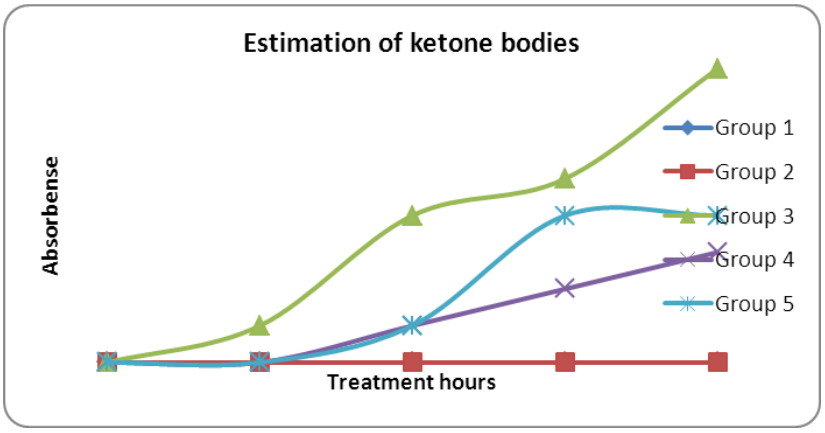

Fig: 1 Estimation of ketone bodies using ethyl acetate extract of *Sesbania grandiflora* leaves

DISCUSSION

The Present study reports the interaction of ethyl acetate extract of *Sesbania grandiflora* leaves on alcohol metabolism. Alcohol is metabolized by the enzyme Alcohol dehydrogenase and is converted to aldehyde in the liver by cytochrome P_{450} 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 *Sesbania grandiflora* at the dose levels of 200mg/kg and 400mg/kg exhibit significant inhibitor of aldehyde dehydrogenase enzyme. The duration of action is 4 hrs which was similar to that of Disulfiram, but found to be less potent inhibitor of aldehyde dehydrogenase enzyme. Further purification and characterization of Ethyl acetate extract of *Sesbania grandiflora* may produce a safer method for de-addiction of alcohol than Disulfiram

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REFERENCES

[1]. Kumar Bishwajit Sutradhar, Naheed Farhana Choudhury. Analgesic and CNS depressant activity of the crude extract of *Sesbania grandiflora*: 1(3), 2009, 56-61.

[2]. Jaideep Singh, Ruchi Singh, Shiv Kumar. Comparing of antioxidant and H2O2 induced free radical scavenging activity of *Sesbania grandiflora* and Acacia nilotica plants: 1(2), 2012.

[3]. E.Harihara Subramanian, Shyju Varghese, N.Rameshkumar, R.Ilavarasan. Pharmacological screening of *Sesbania grandiflora* L.Poiret Extracts: Natural product sciences. 9(3), 2003, 154-157.

[4]. Chandra Sekhar A, Mastanaiah P. Pharmacognistical and Phytochemical screening of *Sesbania grandiflora*: IJARRP. 1, 2013, 1-21.

[5]. Roger Walker, Cate Whittlesea. Clinical Pharmacy and Therapeutics. Edn4, Churchill Livingstone, Elsevier, Philadelphia, USA, 2008, pp.217.

[6]. Bertram G.Katzung. Basic and Clinical Pharmacology. Edn12, McGraw Hill LANGE, NewDelhi, 2012, pp.575.

[7]. Kalpana.B. Munde Wagh, Vijay D. Wagh. Phytochemical, antimicrobial evaluation and determination of total phenolic and flavonoidal contents of *Sesbania grandiflora* flower extract: International journal of pharmacy and pharmaceutical sciences. 4(4), 2012, 229-232.

[8]. Organization for Economic Co-operation and development (OECD) OECD guidelines for testing of chemicals, Toxicity guideline no. 423, acute oral toxicity, fixed dose method.

[9]. Indian Pharmacopoeia (2007). Vol .2007:262

[10]. Organization for Economic Co-operation and development (OECD) OECD guidelines for testing of chemicals, Toxicity guideline no. 407, repeated dose 28 day oral toxicity study in rodents. 2008.