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MODULATORY ROLE OF *Heliotropium indicum* LEAVES ON CYANIDE TOXICITY IN RATS

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ABSTRACT

The present study was designed to investigate the modulatory role of the ethanolic extract of *Heliotropium indicum* leaves. The extract was evaluated by physical parameters as mortality and behavior. The anti-dote activity of *Heliotropium indicum* leaves against potassium cyanide poisoning induced acute poisoning was studied in Wistar albino rats. The leaf extract were pretreated orally (1000 mg/kg) for two days and then treated with potassium cyanide (KCN) at a signle dose of 5mg/kg body weight i.p. (This dose calculated from human dose) (Bhattacharya, 2000). After 12 hrs, the mortality rate and physical observation were monitor and noted. The leaves of *Heliotropium indicum* has possessed modulatory effect against cyanide poisoning.

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INTRODUCTION

Cyanide is regarded as a notorious poison dating back to antiquity. Hydrogen cyanide (HCN) was first isolated from cherry laurel by Swedish chemist, Karl Wilheim Scheele in 1782 and in 1786 he was feared to be the first victim of this rapid poison (Maduh, 1989; Borowitz et al., 1992). Later in 1795 Fontana investigated its mechanism of action, followed by Blakes's attempts to antagonise its toxic effects. However, molecular basis for the biochemical mechanism of cyanide antagonism was first demonstrated in 1933 only (Way, 1984). The ubiquitous existence of cyanide in the environment is associated with the toxic gases produced by pyrolysis of plastic or nitrile based polymer fibres, ingestion of extracts of plants containing cyanogenic glycosides (e.g., cassava) or inhalation from industrial or occupational causes (e.g., electroplating). Administration of certain drugs (e.g., sodium nitroprusside and laetrile) also release cyanide when metabolised in the body. Cyanide poisoning also results from exposure to aliphatic nitrile compounds (e.g. acetonitrile) or by dermal absorption /ingestion of cyanide salts and aliphatic nitriles. Its notoriety as a suicidal, homicidal and genocidal agent is well known (Ballantyne, 1987; Baskin, 1992). Use of HCN as a potent chemical warfare agent is also well documented (Marrs, 1996). Cyanide is a very rapid poison which impairs the cellular respiration leading to a cascade of events culminating in cell death. There are a number of antidotes available for cyanide poisoning. A wide variety of compounds have been used as cyanide antidotes and they have been classified into four major groups based on their mechanism of action: (i)

scavengers, (ii) detoxification, (iii) physiological and (iv) biochemical (Isom GE, Borowitz, 1995).

Scavengers are compounds that inactivate cyanide by binding it or by forming methaemoglobin, which in turn sequesters cyanide. Detoxification agents are listed which enzymatically detoxify cyanide by converting it to a relatively non-toxic product which is readily eliminated from the body. The reaction can be catalyzed by augmenting the levels of the enzyme endogenously or by supplementing the enzyme exogenously or, by providing more substrate to the enzyme, which in this case are sulfur donors (Westley et al., 1983) Oxygen appears to be a physiological antagonist. Oxygen alone at hyperbaric pressure has slight protective effect in cyanide poisoning (Way, 1984). The compounds classified as biochemical antidotes have largely unexplained mechanism of action and are also regarded as non-specific antidotes. These compounds are usually not very effective per se but as adjuncts significantly augment the efficacy of conventional antidotes. A few chemicals Chlorpromazine**,** α-adrenergic blocking agents like phenoxybenzamine (Bhatt and Linnell, 1987).

Traditional herbal medicine is readily available in rural areas for the treatment of insect bite, scorption sting and snake bite. Application of the plant or its sap onto the bite area, chewing leaves and bark or drinking plant extracts or decoctions are some procedure intended to counteract insect bite, scorption sting and snake bite activity. Plants are used either single or in combination as antidotes for insect bite, scorption sting and snake bite by rural populations in India and in many parts of the world. Plants are reputed to neutralize the action of insect bite, scorption sting and snake bite with plethora of plants claimed to be antidotes. Hence, in present study aimed to antidote activity of *Heliotropium indicum* leaves on potassium cyanide poisoning rats. The people always use their own ethnomedicinal plants when they suffer with poisoning (Nadkarni, 2007).

MATERIALS AND METHODS

Animals:

Male albino rats of Wistar strain approximately 3-4 months weighing approximately 140-160g were used in this study. They were healthy animals procured from IAS, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºc and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatization to the environment for 1 week prior to experimental use.

Plant materials

The fully mature *Heliotropium indicum* leaves were collected in April 2013 from Vandayar Iruppu, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Josephs College, Tiruchirappalli, Tamil nadu, India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The collected *Heliotropium indicum* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Heliotropium indicum* leaves extract (HILE) was stored in refrigerator until used. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* antioxidant activity.

Experimental design

Animals were divided into four groups of six rats as follows

Group I Normal

Group II Potassium Cyanide poisoning rats 5mg/kg body weight i.p. (This dose calculated from human dose) (Bhattacharya, 2000).

Group III Pretreated with *Heliotropium indicum* leaves (1000mg/kg) and treated with potassium cyanide (KCN) at a dose of 5mg/kg body weight i.p. (This dose calculated from human dose) (Bhattacharya, 2000)

Group IV Pretreated with known antidote as sodium nitrite (7.5mg/kg) and treated with potassium cyanide (KCN) at a dose of 5mg/kg body weight i.p.

After 12 hrs, the mortality rate and behavior of the animals was monitor and noted up to 24 hrs.

RESULTS AND DISCUSSION

Antidotal activity of *Heliotropium indicum*

Table 1 represents the behavior and mortality rate of experimental rats. The entire Group II rats treated with cyanide poisoning was died at 24 hrs but 2 rats were died and others immobilized at 12 hrs when compared to control rats. Group III and Group IV rats treated with *Heliotropium indicum* extract and sodium nitrite respectively. The frequent movement and improve the intake of food and water were observed in *Heliotropium indicum* extract and standard at 24 hrs. The mortality rate of group II was 100%, Group III was 16.66% and Group IV is 33.33%.

Recently a number of clinical studies suggests that the increase risk of Drug poisoning in Worldwide more than 9 million natural and synthetic chemicals have been identified fewer than 3000 cause more than 95% of accidental and deliberate poisonings all drugs, especially in large doses or when taken over long periods of time, can initiate a toxic condition. Certain drugs or chemicals used in combination, such as alcohol and barbiturates, result in an intensified alteration of physiological state that is frequently dangerous (Vale et al., 2004). Cyanide is ubiquitously present in the environment. It is considered as a potent suicidal, homicidal, genocidal and chemical warfare agent. Cyanide toxicity is mediated through inhibition of cellular respiration, but its other complex toxic manifestations are also well documented. There are a number of antidotes available for cyanide poisoning (e.g.

sodium nitrite, 4-dimethyl aminophenol, sodium thiosulphate, etc.(Bhattacharya, 2000).

Traditionaly *Heliotropium indicum* is used as antidote for snake poison (Nadkarni, 2007) Cyanide acts by binding to the ferric iron in cytochrome oxidase, paralysing energy production throughout the body. However, the ferric iron in methaemoglobin has preferential affinity for cyanide, and combines with it to form cyanmethaemoglobin. This helps to free cyanide from cytochrome oxidase. In order to induce methaemoglobinaemia, nitrites are administered, which oxidize the iron in haemoglobin to produce methaemoglobin (Way, 1984). Iron chelating and iron reducing power activity of *Heliotropium indicum* leaves

Table 1 Behavior and mortality rate of experimental rats.

Parameters	Group I	Group II	Group III	Group IV
Number of rats	06	06	06	06
Mortality	Nil	06	01	02
Behavior of rats (12 hrs)	Normal activities and took food and water	2 rats are died 4 rats were immobilized and decreased intake of food and water	5 rats were immobilized and decreased intake of food and water	5 rats were immobilized and decreased intake of food and water
Behavior of rats (24 hrs)	Normal activities and took food and water	6 rats are died	5 rats were frequent movement and improve the intake of food and water	5 rats were movement and improve the intake of food and water
Number of rats lived	Nil	--	05	04
% of Mortality	--	100	16.66	33.33

were reported our study. This property of *Heliotropium indicum* detoxifies the cyanide. The rich phytochemicals such as flavonoids, terpenoids etc. of *Heliotropium indicum* leaves that inactivate the cyanide by binding it or by forming methaemoglobin, which in turn sequesters cyanide.

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