

Andrographolide Induced Succinate Dehydrogenase Activity in Isolated Mitochondrial Fractions from Different Organ of BALB/C Mice

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Abstract: Andrographolide, a bicyclic diterphenoid lactose, extracted from a plant, *Andrographis paniculata*, is known for its multiple clinical applications in traditional Siddha and Ayurvedic systems in India. Its therapeutic value is perhaps by virtue of its mechanism of action through enzyme induction. The present study is aimed to determine the effects of andrographolide on succinate dehydrogenase (SDH) activity, *in vitro*, using mitochondrial fractions isolated from different organs of BALB/c mice. Administration of andrographolide into mitochondrial fraction of liver, lung and kidney resulted in the induction of SDH. Mitochondrial fraction of lung tissues indicated the maximum SDH acceleratory activity (68.19%), *in vitro*, against 50 µg/ml concentration of andrographolide.

Keywords : Andrographolide, Succinate dehydrogenase, 2,6-dichlorophenol-indophenol, Mitochondrial fraction.

I. Introduction

Andrographis paniculata commonly known as *Nilavembu* is an annual herb, found, in wild, throughout the plains of India especially Tamil Nadu, Karnataka, Maharashtra, Orissa and Uttar Pradesh. The plant species is known for its therapeutic properties viz. Anti-diarrhoeal, anti-inflammatory, anti-choleric, immuno-stimulant and also anti-malarial, anti-hypertensive, antipyretic, anti-thrombotic and hepato-protective (Agrawal, 1997).

Mitochondria enclose the biochemical machinery for cellular respiration; the aerobic processes by which sugars, fatty acids, and amino acids are broken down into carbon dioxide & water and their chemical energy is entrapped as ATP. The Krebs's cycle, also called tri-carboxylic acid or citric acid cycle, is a key series of reactions in this aerobic process. The mitochondrial *succinate dehydrogenase* (SDH) complex catalyses the oxidation of succinate to fumarate in the Krebs's cycle and feeds electrons to the respiratory chain ubiquinone (UQ) pool (Tzagoloff, 1982). Succinate - fumarate reaction is measured by monitoring the reduction of an artificial electron acceptor, 2, 6-dichlorophenolindophenol (DCIP). DCIP is supplied as a dark blue solution and it gets lighter & lighter and finally becomes colourless when reduced. As the solution becomes lighter the absorbance at 600 nm decreases gradually. In order to use an artificial electron acceptor, the normal path of electrons in the electron transport chain (ETC) must be blocked. This is accomplished by adding sodium azide (SA) to the reaction mixture. SA inhibits the transfer of electrons from cytochrome a_3 to the final acceptor, oxygen, so that electrons cannot be passed.

II. Material and Method

1: Cervical dislocation (CD) euthanasia and isolation of mice organs

Six-week old male BALB/c mice were euthanized by cervical dislocation. The thumb and index finger were placed on either side of the neck at the base of the skull or a narrow, blunt instrument, such as the dull edge of a scissor blade, acrylic ruler or cage card holder, was pressed at the base of the skull. With the other hand, the base of the tail or the hind limbs were pulled quickly, causing separation of cervical vertebrae from the skull.

2: Separation of mitochondria by cellular fractionation

Cellular fractionation of mice homogenized liver, lung and kidney were accomplished through differential centrifugation.

3: Extraction and Screening of andrographolide

The plant, *Andrographis paniculata*, was collected from nursery. The leaves were shade-dried, followed by blowing in hot air oven for 30 minutes at low temperature and then powdered. The dried powder (100 gm) was subjected to extraction with 1200 ml ethanol for 48 hr. The ethanol extract was collected, filtered & concentrated in vacuum under reduced pressure and dried in desiccators. Employing thin layer chromatography (TLC), 5 ml of the ethanolic leaf extract was put on each spot. A precoated plate of silica gel 60F254 (Merck)

and mobile phase (chloroform: methanol: ethyl acetate - 8.0 : 1.5 : 1.0) were used. The extracts, fractionated by TLC, were detected in UV radiation (Electronic UV Transilluminator, Quantum Scientific). The R_f of andrographolide was 0.58.

4 : Screening for enzyme inducing effect of andrographolide

Different concentrations of *andrographolide* (10-50 mg/ml) were used for the screening of SDH activity. These concentrations were added with mitochondrial fractions (excised from liver, lung and kidney) DCIP and SA. The experiment was carried out with two separate controls: 1) no SA (ensuring normal electron flow through the respiratory chain together with electron flow to DCIP) and 2) no *andrographolide* (ascertaining positive control for normal optimum activity of SDH).

5: Determination of SDH activity

The reaction was measured at 600 nm using spectrophotometer. An electron acceptor was introduced, which accepted the electrons of FADH₂ and got reduced. In order to ensure one way flow of electrons, the normal path of electron transfer, through the respiratory chain, was blocked using SA. It blocked the transfer of electrons from cytochrome a₃ to molecular oxygen, bringing the entire process of electron transfer in the respiratory chain to a standstill. All the electrons generated from the oxidation of succinate then flowed to the introduced electron acceptor.

Results & Discussion

SDH acceleration studies of bioactive compound *andrographolide* against mitochondrial fractions of kidney, liver and lung tissues are presented in Table - 1. The hastening effect of *andrographolide* on SDH activity in lung fraction was relatively more prominent than on SDH activity in mitochondrial fractions taken from kidney and liver. At 50 µg/ml concentration, it was 68.19%, 59.52% and 59.11% for liver, lungs and kidney respectively. During positive control (when no *andrographolide* was administered) SDH activity was found to be 38.50%, 57.66% and 54.63%.

Table - 1 : Effect of *Andrographolide* on SDH activity in mitochondrial fractions of liver, lungs and kidney using Spectrophotometer.

SDH activity	Concentration of andrographolide (µl/ml)					
	Positive Control	10	20	30	40	50
Mitochondrial fractions						
Kidney	0.225	0.210	0.195	0.172	0.112	0.092
Liver	0.210	0.190	0.180	0.165	0.095	0.085
Lungs	0.305	0.265	0.211	0.192	0.173	0.102

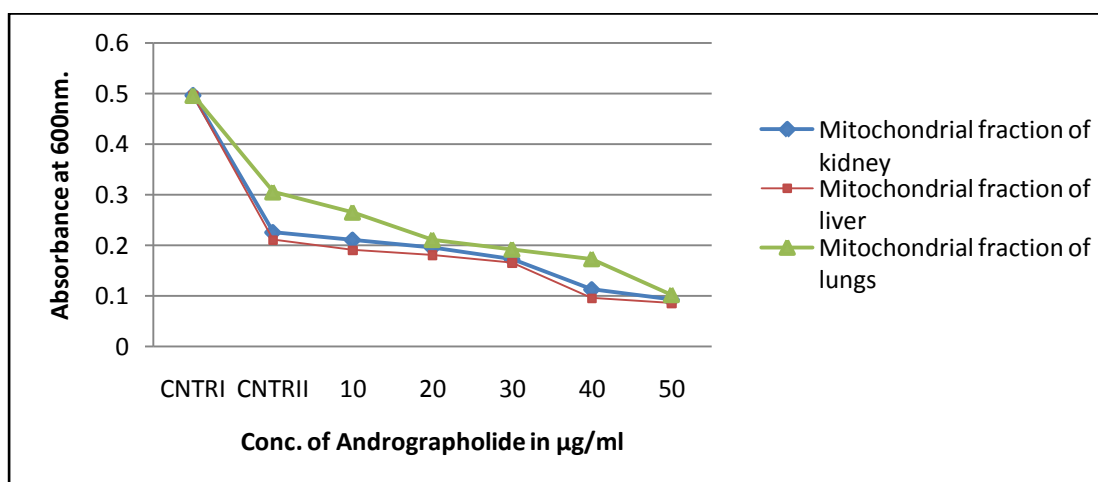


Fig - 1 : Effect of *Andrographolide* on SDH activity in mitochondrial fractions of liver, lungs and kidney.

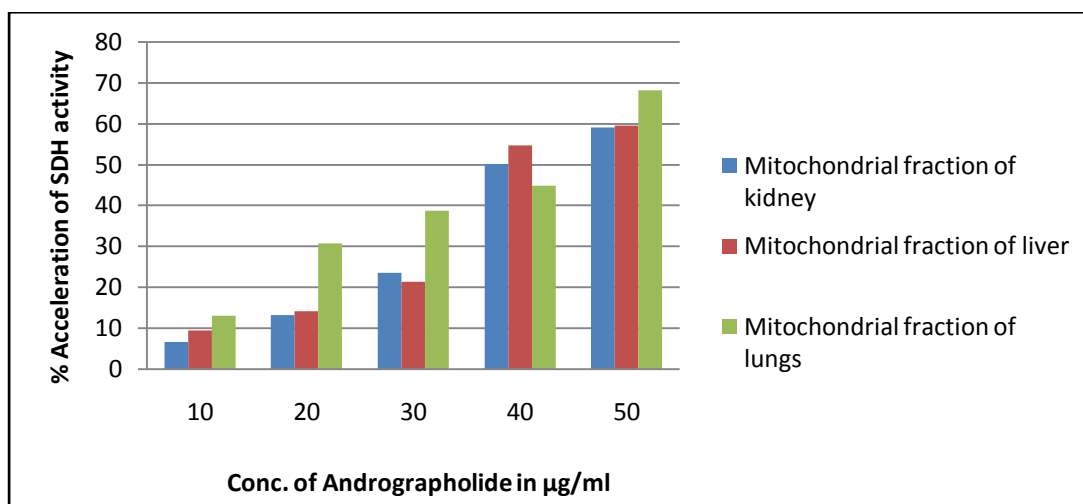


Fig - 2 : Percentage Acceleration in SDH activity

SDH apart from playing a central role in the Krebs's cycle and the respiratory chain, also outdoes other mitochondrial dehydrogenases owing to its exclusive redox properties. In association with ubiquinone, SDH represents a crucial antioxidant enzyme in mitochondria, controlling superoxides scavenging activity in its respiratory chain. Recent studies have revealed that SDH genes act as a tumour suppressor in humans (Tomlinson *et al.*, 2002). During present study, andrographolide induced SDH activity against the mitochondrial fractions of liver, kidney and lung tissues showed substantive acceleration. The bioactive compound, andrographolide, is a herbal medicine and has been used in the treatment of diverse cellular and metabolic disorders including cancer. (Parfait *et al.*, 2000).

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