

## **IN VITRO METABOLIC STABILITY OF MONOCROTALINE (MCT) AND HELIOTRINE (HLT) IN RAT LIVER MICROSOME**

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### **ABSTRACT**

The Pyrolizidine alkaloids (PAs) monocrotaline, and heliotrine chosen in our investigation are important as there are many reports if their occurrence as constituents of various food components as contaminated to cereal crops and herbal dietary supplements. These two PAs also belong to the two major classes of tumorigenic PAs, which is retronecine and heliotridine class. Although there is a sizable literature on monocrotaline and heliotrine, the information on the metabolism and toxicity of the important components of comfrey, is quite limited. Metabolic stability is defined as the percentage of parent compound lost over time in the presence of a metabolically active test system. The study in the compounds (MCT and HLT) stability is very important in order to investigate the profile of our compounds and as a foundation for further experiment. By understanding the metabolic stability of MCT and HLT in the early experiment will able to rank them in term of their rates of metabolism (slow or fast metabolism) and also to determine their half –life that will be benefit on define the incubation & sampling time. The aim of this research is to determine Metabolic Stability of Monocrotaline (MCT), and Heliotrine(HLT) in rat Liver Microsome. Rat liver microsome extraction and Higher Throughput Screening for Metabolic Stability.

This research suggest that the two compounds of PAs Monocrotaline and Helitorine have demonstrated the stability in the liver microsome compare to propranolol . Their slowly metabolism in the liver or longer half-lives may explain their high properties to survive long enough to reach target organs and their metabolites to bind with tissue and macromolecule.

Keywords: heliotrine, metabolic stability, monocrotaline

## INTRODUCTION

Plants containing Pyrrolizidine Alkaloids (PAs) widely distributed in the world have been a risk to human health since they possess significant hepatotoxic, pneumotoxic, genotoxic and carcinogenic effects to animal experiments and poisoning to humans (Chan *et al.*, 1994); (Cheek, P.R., 1988). More than 660 pyrrolizidine alkaloids have been identified in over 6000 plants of three most toxic PAs families and about half of them exhibit toxic activities (Dubecke & Lullman, 2011).

Monocrotaline found in *Crotalaria sp.*, commonly known as *rattlepods* is derived from the fact that the seeds become loose in the pod as they mature, and rattle when the pod is shaken. As a legume *Crotalaria* species supports nitrogen fixing bacteria, and it is considered as a soil builder and soil fertilizer<sup>1</sup>. They grow widely in pastures in Northern Australia and Western Australia and poisoned grazing livestock due to the alkaloid Monocrotaline a toxic PAs contain in them. Heliotrine present in *Heliotropium europaeum*, a summer growing weed, is responsible for large number of deaths in sheep in parts of southeastern Australia<sup>2,3,4</sup>. Several *Heliotropium sp* mostly known as a garden plants, however they contain *heliotrine* a toxic PAs and some species are weeds. Continual ingestion by livestock of large amounts of heliotrope plants (either fresh or dried), or of their seeds as contaminants in stock feed, can cause hepatotoxicity and reduced productivity. Horses, pigs, cattle, sheep and goats can be all affected, but display decreasing susceptibility with horses being the most susceptible<sup>5</sup>. It has been shown by Bull *et al.*, (1956) that sheep grazed on *Heliotropium europaeum* for two or more consecutive summer seasons, produce a chronic liver disease. Metabolic stability is defined as the percentage of parent compound lost over time in the presence of a metabolically active test system. The study in the compounds (MCT and HLT) stability is very important in

order to investigate the profile of our compounds and as a foundation for further experiment.

## METHODS

### Rat liver microsome extraction

Method according to Abernathy *et al.* Male Wistar rat 250 g, BRF was killed by carbon dioxide. The livers were perfused with ice cold saline to remove excess blood and homogenised in four volumes of ice-cold 0.1M potassium phosphate buffer (pH 7.4). The homogenates were centrifuged using Beckman Avanti J-25 Centrifuge at 9000x g for 20 minutes, and the supernatant was collected. The supernatant fraction was re-centrifuged using Beckman Optima MAX-XP Ultracentrifuge at 105000x g for 60 minutes, then the pellet was collected. The microsomes obtained were re-suspended in 0.01M ice-cold potassium phosphate buffer pH 7.4 containing 0.1mM EDTA and 20% glycerol and determine the protein concentration. Adjust to 5 mg/ml, make a batch by transferring it into the eppendorf tube and store at -80°C prior to use.

### Higher throughput screening for metabolic stability

Method according to Jeanette R. Hill<sup>8</sup> with modification. Make a 100 µM stock of Monocrotaline (MCT), Heliotrine (HLT), and Propranolol (PPL) in Phosphate Buffer pH 7.4. Mix the formula of 175 µL Phosphate Buffer, 25 µL NADPH, 25 µL microsome calculate based on the amount of compounds and replication we used. Transfer them into 96-wells Incubation Plate. In another 96-wells, compound Plate fill in 250 µL MCT, HLT, PPL. Then incubate the Compound Plate and Incubation Plate for 5 minutes in the shaker incubator 100 rpm at 37°C. To separate 96-wells, Quenching Plate adds 50 µL of ice cold methanol and store at 4°C. The reaction was initiated by adding 25 µL of compounds to Incubation Plate and gently mixed them by pipetting up and down.

Then immediately remove 100  $\mu\text{L}$  to the Quenching Plate for 0 minute time. Add 25  $\mu\text{L}$  of MCT, HLT, PPL to the rest of the Incubation Plate for 5, 10, 30, and 60 minutes time point sampling.

**RESULT AND DISCUSSION**

The stability of monocrotaline and heliotrine at 1 hour has shown in Table 1.

Table 1. The stability of monocrotaline and heliotrine in rat liver microsome

<b>Compounds</b>	<b>T <math>\frac{1}{2}</math> (minutes)</b>	<b>% Parent compound turnover at 1 hr</b>
Monocrotaline	133.27	25.35
Heliotrine	144.38	21.55
Propanolol	17.10	91.43

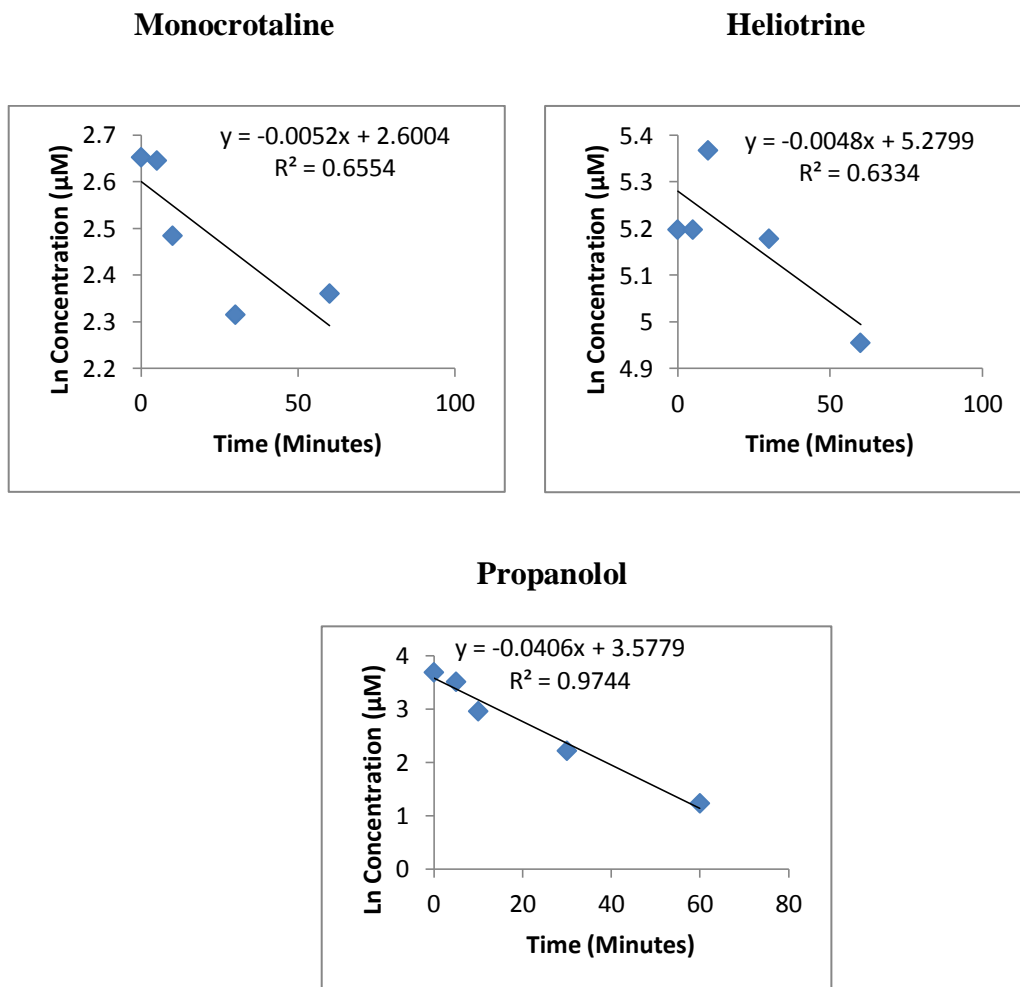


Figure 1. In vitro metabolic stability of monocrotaline and heliotrine in rat liver microsome with propanolol as a positive control

The three compounds of PAs Monocrotaline and Heliotrine, have demonstrated the stability in the liver microsome compare to propranolol as shown on table 1 and figure 1. Their slowly metabolism in the liver or longer half-lives may explain their high properties to survive long enough to reach target organs and their metabolites to bind with tissue and macromolecule. A monocrotaline kinetic study by Estep et al., also support this longer half-lives data, particularly monocrotaline. They show the elimination of radiolabel monocrotaline in the urine at 7 hr after injection to the rat approximately 90% recovery of the parent compound<sup>9</sup>. There is not much data reported in heliotrine kinetic study, but from the data we obtained suggest that both of them are stable in the liver microsome. There is not much data reported in heliotrine and kinetic study, but from the data we obtained suggest that both of them are stable in the liver microsome.

Metabolic stability is defined as the percentage of parent compound lost over time in the presence of a metabolically active test system. Since liver is the main organ in metabolism, especially for the phase I metabolism; where the main metabolizing enzymes CYP-450 and their isozyme are located within the endoplasmic reticulum in the liver. Hence, liver microsome will use in this experiment to investigate the phase I metabolism pathway in MCT and HLT. The study in the compounds (MCT and HLT) stability is very important in order to investigate the profile of our compounds and as a foundation for next experiment. By understanding the metabolic stability of MCT and HLT in the early experiment, we will able to rank them in term of their rates of metabolism (slow or fast metabolism) and also to determine their half –life that will be benefit on define the incubation & sampling time.

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