UNIVERSITI PUTRA MALAYSIA

TOXIC EFFECTS OF SIGNAL GRASS (*BRACHIARIA DECUMBENS*) ON DRUG- METABOLIZING ENZYME ACTIVITIES IN SHEEP

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

July 2003

" To my

late father, Hussain bin Hassan,

beloved mother, Salimah binti Ismail

brother and sisters,

wife and children,

and all those individuals,

whom, without their continuous encouragement and motivation

I may not be able to complete this study"

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Professor Dato' Abdul Salam Abdullah, Ph.D.

Faculty : Medicine and Health Sciences

Signal grass (*Brachiaria decumbens*) is widely grown on livestock farms in many countries including Malaysia due to its high productivity and nutritive value. Unfortunately, it is toxic to sheep and goats causing a severe hepatic and renal damage, and death. Two experiments were conducted to determine the effect of the toxicity of signal grass (*B. decumbens)* on the activity of drug-metabolizing enzymes (DME) in sheep and cattle. It was hypothesized that the activities of selected enzymes would be affected during intoxication and that differences in the activity level of the enzymes in the two species might explain why cattle were safe from the toxic effect of the grass. Twenty-three healthy Wiltshire-Malin crossed rams, aged 14-16 months were used, fifteen for the first experiment and eight for the later. In the first experiment, aniline 4-hydroxylase (A4H), aminopyrine N-demethylase (AND), UDP-glucuronyl transferase (UDPGT), glutathione S-transferase (GST) and cytochrome P450 of the liver and kidney of the control and intoxicated sheep were

determined *in vitro*. Activities of DMEs in normal cattle and normal sheep were also compared. In the second experiment, the pharmacokinetics of intravenously administered sulphadimidine (100 mg/kg) and antipyrine (20 mg/kg) were determined, prior to introduction to this toxic grass and after the toxicity signs developed. Statistical analysis was done by using ANOVA and Student's t-test, and pharmacokinetic parameters were subjected to Mann-Whitney Rank Sum Test. The concentration of cytochrome P450 was found to be higher in the liver of intoxicated group, and lower in the kidney, compared to the control group. However, the activities of the four enzymes in both the liver and the kidney were generally lower in the intoxicated group. In comparison with cattle, the concentration of cytochrome P450 was higher in the liver of cattle but lower in the kidney of sheep. The activities of AND, A4H, UDPGT and GST were higher in the liver (except GST) and kidney of the sheep than in the cattle. In the pharmacokinetics study, there was a decrease in the total body clearance, Cl_t, and this prolonged the elimination half-life ($t_{1/2}\beta$) for both drugs. No changes were observed in the volume of distribution of antipyrine before after being intoxicated. For sulphadimidine, the amount of drug retained in the body increased. This study demonstrated that there was an impairment of hepatic and renal DMEs activities *in vitro* and *in vivo* in the intoxicated sheep. The resistance to signal grass observed in cattle was not likely due to the effective protective mechanism of hepatic and renal detoxification, since their DMEs activities were actually lower than those recorded in sheep.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah master

KESAN KERACUNAN RUMPUT SIGNAL (*Brachiaria decumbens*) KE ATAS AKTIVITI ENZIM MEMETABOLISMA DRUG DALAM BIRI-BIRI

Oleh

MOHD KHAIRI BIN HUSSAIN

Julai 2003

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Rumput Signal (*Brachiaria decumbens*) ditanam secara meluas di ladang ternakan di kebanyakan negara termasuk Malaysia kerana nilai pemakanan dan hasil pengeluaran yang tinggi. Malangnya, ia beracun kepada biri-biri dan kambing menyebabkan kerosakan teruk hati dan ginjal, dan kematian. Dua ujikaji telah dijalankan untuk menentukan kesan keracunan rumput Signal ke atas aktiviti enzim memetabolismakan drug (DME) di dalam biri-biri dan lembu. Hipotesis kajian ini, aktiviti enzim-enzim yang dipilih akan terganggu semasa mengalami keracunan dan perbezaan di dalam aktiviti DME di antara dua spesis mungkin boleh menerangkan ketahanan lembu terhadap keracunan rumput ini. Dua puluh tiga ekor biri-biri jantan dari baka kacukan Wiltshire-Malin, berumur 14-16 bulan digunakan, 15 untuk ujikaji pertama dan lapan untuk yang berikutnya. Di dalam ujikaji pertama, aktiviti enzim-enzim aniline 4-hidroksilase (A4H), aminopirine N-demetilase (AND),UDP-glukuronil transferase (UDPGT), glutathione S-transferase (GST) dan sitokrom P450

di dalam hati dan ginjal biri-biri kawalan dan yang keracunan telah ditentukan secara *in vitro*. Aktiviti enzim-enzim ini juga dibandingkan di antara lembu dan biri-biri yang sihat. Di dalam ujikaji kedua, penentuan farmakokinetik sulfadimidin (100 mg/kg) dan antipirin (20 mg/kg) yang diberi secara intravenus telah dijalankan iaitu sebelum pendedahan kepada rumput beracun ini dan selepas menunjukkan tanda-tanda klinikal keracunan. Analisa statistik telah dibuat dengan menggunakan ANOVA dan ujian t Student, dan parameter farmakokinetik ditambah dengan Ujian Pangkat Mann-Whitney. Kepekatan sitokrom P450 adalah lebih tinggi di dalam hati kumpulan yang mengalami keracunan, dan lebih rendah di dalam ginjal berbanding kumpulan kawalan. Walau bagaimanapun, aktiviti keempat-empat enzim di dalam hati dan ginjal secara amnya lebih rendah kumpulan yang mengalami keracunan. Dibandingkan dengan lembu, paras sitokrom P450 lebih tinggi di dalam hati lembu tetapi lebih rendah di dalam ginjal biri-biri. Aktiviti keempat-empat enzim adalah lebih tinggi di dalam hati (kecuali GST) dan ginjal biri-biri berbanding lembu. Daripada kajian farmakokinetik, terdapat penurunan dalam penyingkiran total badan, Cl_t, dan ini memanjangkan separa hayat eliminasi ($t_{1/2}\beta$) untuk kedua-dua drug yang dikaji. Tiada perubahan dalam isipadu penyebaran drug di dalam biri-biri sebelum dan selepas keracunan. Kuantiti sulfadimidin yang dikekalkan di dalam badan meningkat seperti yang digambarkan oleh peningkatan luas di bawah keluk, AUC. Kajian ini menunjukkan berlakunya perencatan di dalam akitiviti DME secara *in vitro* dan juga *in vivo* di dalam hati dan ginjal biri-biri yang mengalami keracunan. Ketahanan lembu terhadap rumput Signal bukanlah disebabkan perlindungan oleh mekanisme detoksifikasi hati dan ginjal, kerana aktiviti DME lembu adalah lebih rendah berbanding biri-biri.

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I certify that an Examination Committee met on 11th July 2003 to conduct the final examination of Mohd Khairi Hussain on his Master thesis entitled " Toxic Effects of Signal Grass (*Brachiaria decumbens*) on Drug-Metabolizing Enzyme Activities in Sheep " in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master Science. The members of the Supervisory Committee are as follows :

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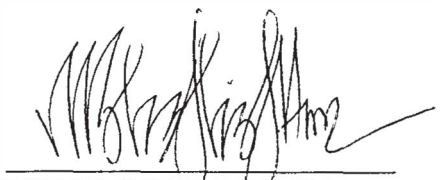
Date : 14 NOV 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



MOHD KHAIRI HUSSAIN

Date : 14th August 2003

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

mM	millimolar
M	Molar
µl	microliter
l	liter
µ mole	micromole
µg	microgram
kg	kilogram
g	gram
mg	milligram
mg/kg	milligram per kilogram
g	gravity force
w/v	weight per volume
v/v	volume per volume
min	minute
sec	second
N	Normal
et al.	*et alia*
nm	nanometer
°C	degree Celcius

CHAPTER 1

INTRODUCTION

Signal grass (*Brachiaria decumbens*) is widely grown in livestock farms in many countries including Malaysia. It is an important source of fodder for ruminants due to its high productivity and nutritive value (Chin *et al*., 1979) and it is well adaptation to the tropical climate (Loch, 1977). Unfortunately, is toxic to sheep and goats causing a severe hepatotoxic and nephrotoxic damage and death (Abas Mazni *et al.*, 1983; Salam Abdullah *et al*., 1987; Salam Abdullah *et al*., 1989; Noordin *et al*., 1987).

The first case of *B. decumbens* toxicity in Malaysia was observed in cattle in 1975, which affected a few breeds of cattle (Abas Mazni and Sharif, 1986). In 1979, cases of hepatic jaundice and photosensitization were observed in sheep and goats at the Malaysian Agriculture Research and Development Institute (MARDI), Serdang (Suparjo and Wahid, 1980). In the following year, another incidence of *B. decumbens* toxicity occurred in sheep 10 days post grazing (Sharif *et al*., 1985). The incidence declined after the animals were removed from the *B. decumbens* pasture (Abas Mazni and Sharif, 1986).

Such incidence occurred quite extensively in the government farms affecting mainly sheep and goats with high mortalities (Abas Mazni and Sharif, 1986; Shahirudin *et al*., 1983; and Sharif *et al*., 1985). However, goats were found to be less affected and

required a longer period of grazing to be intoxicated compared to sheep (Abas Mazni, 1985).

Attempts to overcome the problem as well as to improve the quality of the grass have been carried out. For example, supplementation of zinc sulphate in drinking water was found to provide quite a significant degree of protection from the toxic effects of this grass. However, it was observed that the supplement was not palatable to the animal (Salam Abdullah *et al*., 1994).

Drug metabolism or biotransformation plays an important role in determining the fate of xenobiotics in the animal body from the point of administration until elimination or excretion. Changes in the activities of drug metabolizing enzymes may lead to various physiological, pharmacological or toxicological consequences. There is little or no information with regards to the toxic effects of *B. decumbens* on the activities of drug-metabolizing enzymes, which represent important oxidative and conjugative metabolic pathways. Four drug-metabolizing enzymes were chosen namely aminopyrine N-demethylase and aniline 4-hydroxylase (represented the oxidative enzymes), UDP glucuronyl transferase and glutathione S-tranferase (represented the conjugative enzymes). This four enzymes are among the routinely used in laboratory experiments in the study of drug-metabolizing enzymes (La Du *et al*,. 1971)

The enzymatic activities in sheep were compared to cattle, which appeared to be resistant to this grass toxicity (Nordin *et al*., 1989). It is widely recognized that species

differences in biotransformation of drug play a significant role in determining susceptibility to a drug or poison.

In *B. decumbens* intoxicated animals, the liver and the kidneys were severely affected or damaged organs (Noordin *et al.*, 1987; Zamri-Saad *et al.*, 1987). As the activities of drug-metabolizing enzymes are affected, it is important that the pharmacokinetic of therapeutics drugs during the period of intoxication is assessed, as the dosage may need to be modified accordingly.

Knowledge of drug metabolism especially the hepatic microsomal drug metabolizing systems is important in determining the resistance of animals to drugs and toxins (Parke, 1976; Seawright *et al.*, 1972) especially in animals having a liver disease. Thus, the investigation on the effect of the toxic substances on the changes of drug metabolizing enzyme activities is essential.

As the liver and kidneys play an important role in drug metabolism, it is hypothesized that the drug-metabolizing enzyme activities in sheep intoxicated with *B. decumbens* will be affected and later alter the pharmacokinetic parameters. Another hypothesis is there are differences in the activity level of the enzymes in the two species (sheep and cattle) that might explain why cattle were safe from the toxic effect of the grass.

Knowledge obtained from this study could help in future planning on reducing or overcoming the toxicity of *B. decumbens* in sheep, thus increasing sheep production to cater for the need of the country.

The objectives of this study were:

1. to investigate the effects of the Signal grass (*B. decumbens*) toxicity on the activities of drug metabolizing enzymes in sheep
2. to study the pharmacokinetics of selected chemotherapeutic agents in sheep intoxicated by *B. decumbens*
3. to compare the activities of drug metabolizing enzymes between normal sheep and cattle

CHAPTER 2

LITERATURE REVIEW

2.1 Drug Metabolism

Drug metabolism is defined as the sum of the processes affecting the fate of drugs and other xenobiotics in the organism (Mandel, 1971). Drug biotransformations are the metabolic changes of drugs and other organic compounds foreign to the body, through a system of enzymes of low substrates specificity requirements, *in vivo*, to produce metabolic products. The latter which are more polar and less lipid soluble than the parent compound, have diminished their activity or are inactive, and then are readily excretable into the bile and urine (Correia, 2001).

Drug metabolizing systems therefore, are very important for the elimination of potentially toxic compounds from the body (Williams, 1972; Gram and Gillette, 1975). Brodie (1964) noted that if there were no such processes as drug metabolism, it would take the body about 100 years to terminate the action of pentobarbital, which is lipid soluble and thus cannot be readily excreted without being metabolized. This statement manifests the significance of drug metabolism.

The process of drug metabolism has been traditionally classified as Phase I and Phase II only, and Phase III was later included in the last two decades. Phase I serves to

unmask a functional group and involved oxidation, reduction and hydrolysis. For example, benzene being hydrophobic can partition into the cell membrane and change its properties, but its toxicity is limited without further metabolism (Zimniak *et al.*, 1999). Phase II reaction is the conjugation of the metabolite with one of a number of moities such as glucuronic acid, glutathione and sulfate. For conjugation to occur, an appropriate acceptor group must be present in the compound. Many xenobiotics lack such functional polar groups and require metabolic activation mediated by phase I enzymes before becoming acceptors for phase II reaction. Usually conjugation adds to the bulk of the molecule, increases its water solubility and decreases its reactivity, making the molecule less toxic.

It was initially thought that Phase II reactions complete the processes begun by Phase I transformations. This appears to be incorrect as certain compounds are activated, rather than detoxified, even by successive Phase I and Phase II reactions (Anders and Dekant, 1998). In certain cases, the metabolite retains some reactivity making the metabolism pathway incomplete. The anticancer drug, thiotepa, whose glutathione conjugate retains DNA alkylating properties due to presence of second functional group (Cnubben *et al.*, 1998). In addition the glutathione conjugates can inhibit glutathione S-tranferase, an important detoxifying agent (Awasthi *et al.*, 1993). Conjugation with a highly polar moiety such as glutathione traps the reaction product within the cell by preventing its diffusion across the biological membranes. Continiuing accumulation of conjugates will eventually become detrimental to the cell. The acetyl metabolites of

sulfapyrine and sulfamerazine are very water-insoluble causing kidney damage resulting from intratubular crystallization (Williams, 1959).

The elimination of the products of these reactions from cells is thus an integral part of the detoxification process which can be achieved through further metabolism. The removal of the products from the cell through an active transport has been termed as Phase III reaction which is not only energy-dependent, but also directly coupled to the hydrolysis of ATP (Ishikawa, 1992).

2.1.1 Sites of Drug Metabolism

The liver is the largest visceral organ in the body and forms about 3-5% of body weight. It plays multi-roles including excretion, storage and homeostasis as well as the maintenance of constancy in the internal environment of the body (Andrew, 1979). One major role played by liver is drug biotrasformation or drug metabolism. Even though drug-metabolizing activities have been identified in the adrenal gland, the intestinal mucosa, lung, kidney, blood plasma and at to a certain extent by the gut microflora it is the liver that performs the largest share of drug metabolism (Gram and Gillette, 1975).

The enzymes involved in drug metabolism termed as drug-metabolizing enzymes are found mostly in the endoplasmic reticulum, the cytosol (soluble or cell sap fraction) and mitochondrial fractions of the hepatocytes (Mandel, 1971). Endoplasmic reticulum in an intact cell which consists of continuous filamentous membrane bound channels, and

upon homogenization it is physically disrupted, but it retains most of the enzymatic activities in small vesicles termed microsomes (Gibson and Skett, 1986).

2.1.2 Fates of Drugs After Metabolism

After going through the metabolic processes, the activity of a drug is altered as follows:

a. Transformation of inactive drugs or "pro-drug" into pharmacologically active drugs, or an active drug into a more active metabolite. For example, chloral hydrate is converted into trichloroethanol, demethylation of imipramine to desmethylimipramine and the oxidation of diethylene glycol to oxalate (Mandel, 1971).

b. Transformation of a drug into another compound with a qualitatively similar or different pharmacological activity. For example, phenacetin is oxidised into paracetamol; daizepam is converted into N-desmethyldiazepam and acetylsalicylic acid converted into salicylic acid (Williams, 1972).

c. Convertion of a drug into a relatively inactive drug. For example, procaine is metabolised into p-amino benzoic acid and griseofulvin into 6-demethylgriseofulvin.

In certain cases, the drug is excreted unchanged without undergoing any biotransformation.