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# Assessment of Tonica, an Aqueous Herbal Haematinic, in the Modulation of Rat Hepatic Microsomal CYP-Mediated Drug Metabolizing Enzymes: Implications for Drug Interactions

[1]O.N.K. Martey, [2]A. Ocloo, [2]E. Koomson and [1]L.K.N. Okine
[1]Centre for Scientific Research into Plant Medicine,
P.O. Box 73, Mampong, Akuapem, Ghana
[2]Department of Biochemistry, P.O. Box LG 56,
University of Ghana, Legon, Ghana

**Abstract:** The effects of Tonica (TN), an herbal haematinic prepared from the stem barks of *Khaya senegalensis*, *Mitragyna stipulosa* and *Kigelia africana*, on the activities of hepatic microsomal cytochrome P450 (CYP) enzymes were investigated in Sprague-Dawley rats. TN was administered to rats, by oral gavage, at the normal human dose (28 mg/kg/day), 10x and 20x that dose for 6 weeks. Activities of certain hepatic CYP drug-metabolizing enzymes and pentobarbital-induced sleeping time were determined in control and TN-treated animals. There were insignificant ($p>0.05$) increases in the microsomal protein content (3.25-31%) at all doses of TN in a non-dose-dependent fashion. However, there was a general insignificant attenuation of NADPH cytochrome c ($P_{450}$) reductase activity in TN-treated animals compared to control (8.9-26.1%). p-Nitrophenol hydroxylase (pNPH) activity was insignificantly ($p>0.05$) elevated (14.8-23%) in the TN-treated rats compared to control. The activities of aminopyrine-N-demethylase (AmD) and nitroanisole-O-demethylase (NOD) at the normal and 10x the normal dose of TN were not significantly different from controls, but at 20x the normal dose these enzyme activities were insignificantly ($p>0.05$) elevated above controls (11.7 and 39.8% for AmD and NOD, respectively). Pentobarbital-induced sleeping time in TN pre-treated animals were insignificantly ($p>0.05$) inhibited compared to control (3.7-9.5%). These results suggest that TN by insignificantly elevating certain CYP isozymes may have the potential of modulating the metabolism of substances other than pentobarbital.

**Key words:** *Khaya senegalensis*, *Mitragyna stipulosa*, *Kigelia africana*, cytochrome P450, pentobarbital

## INTRODUCTION

The patronage of herbal medicine is progressively rising in the world today (Lazarou *et al.*, 1998). People use herbs mainly as food and for therapeutic purposes with or without medical prescription. Regarding therapy, a patient often consumes two or more drugs concurrently and this is capable of engendering drug-drug interaction. Herbal medicines are coming under increasing attack for being dangerous to patients, especially those on multiple medications (Starfield, 2000).

Tonica (TN) is a herbal haematinic prepared from the stem barks of *Khaya senegalensis*, *Kigelia africana* and *Mitragyna stipulosa* (Mshana *et al*., 2000) by the Centre for Scientific Research into Plant Medicine (CSRPM) and is effective for restoration of appetite and a boost in haemoglobin

**Corresponding Author:** Laud K.N. Okine, Centre for Scientific Research into Plant Medicine, P.O. Box 73, Mampong-Akuapem, Ghana Tel: 233-24-334-0816 Fax: 233-872-22103

levels in anaemic patients (Adusi-Poku *et al*., 2008). It contains reducing sugars, saponins and polyuronides as groups of phytoconstituents.

The stem bark of *Khaya senegalensis* (Meliaceae) has been shown to potentially treat malaria, convulsion, arthritis, haemorrhoids and anaemia (Mshana *et al*., 2000; Ademola *et al*., 2004; Egesie *et al*., 2004; Androulakis *et al*., 2006). *Kigelia africana* (Bignoniaceae) is noted for its use in treating constipation, lumbago, snakebite and haemorrhoids from the root or fruit preparation. The bark and the leaves of the plant are, respectively, important in the remedy of arthritis, anaemia and wounds (Akunyili *et al*., 1991; Mshana *et al*., 2000). Finally, *Mitragyna stipulosa* (Rubiaceae) has been reported to be therapeutic for malaria, loss of appetite, inflammations, hypertension, headache, rheumatism, gonorrhea, broncho-pulmonary disease and paralysis (Mshana *et al*., 2000; Dongmo *et al*., 2003).

Conventionally, drug metabolism is broadly divided in phase I and phase II processes (Meech and Mackenzie, 1997; Woolf, 1999). Cytochrome $P_{450}$ (CYPs) are involved in the metabolism of various xenobiotic and endogenous compounds (Backes and Kelly, 2003). Human CYP isoforms that are involved in the biotransformation of xenobiotics include CYP1A/2, CYP2B6, CYP2C8/9/19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A (Shimada *et al*., 1994; Woolf, 1999).

Adverse drug interactions are sometimes life threatening and these can be minimized when medications are administered in the right doses and by understanding the mechanisms of drug-drug interactions. Bleeding is enhanced when warfarin, an anticoagulant is combined with garlic (*Allium sativum*), dong quai (*Angelica sinensis*) or danshen *(Sulvia miltiorrhiza*) (Adriane, 2000). Finally Trikatu, a commonly used herb in Ayurvedic medicine can markedly reduce the peak concentration of rifampicin by slowing absorption (Karan *et al*., 1999).

Tonica is most often administered to pregnant women and patients suffering from malaria. Hence there is the potential of drug interactions with other herbal or orthodox medicines as a result of its co-joint administration with these medicines. Therefore, in the present study the potential of TN for drug interaction via drug metabolism was assessed by studying its effects on selected CYP-dependent microsomal enzyme activities and pentobarbital-induced sleeping time.

## MATERIALS AND METHODS

### Chemicals and Reagents

Glycerol, sucrose, Coomassie brilliant blue (G-250), p-nitrophenol, nicotinamide adenine dinucleotide phosphate reduced (NADPH), bovine serum albumin (BSA), cytochrome c, perchloric acid ($HClO_4$), 1-aminopyrine, semicarbazide HCl, Nash reagent, 4-nitroanisole, Trizma base and formaldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA) was obtained from Timstar Laboratory Suppliers Ltd., UK.

### Preparation of Tonica Extract

Tonica was prepared from the stem barks of three different plant species: *Khaya senegalensis, Mitragyna stipulosa* and *Kigelia africana* in kilogram quantities (8:4:2 w/w) in 110 L of sterile distilled water. This was boiled for 1 h, cooled to room temperature and re-boiled for another hour. The extract was sieved and freeze-dried (Heto Power dry LL 3000, Denmark) to give a dry extract yield of 72000 mg $kg^{-1}$ plant raw material (7.2% w/w) and stored in a cool dry place. The pharmaceutical quality control of TN extract involved organoleptic, microbial and physicochemical analysis as well as quantitative determination of saponins, total solid residue and total extractives. The extract was reconstituted in sterilized distilled water before administration to animals.

### Experimental Animals

Age-matched Sprague-Dawley rats (150-250 g) were obtained from the Animal Unit of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong, Akuapem, Ghana. The animals were

fed on standard laboratory chow obtained from Ghana Agro Food Company (GAFCO), Tema, Ghana and water *ad libitum*. They were acclimatized for a week before administration of TN extract. Studies were conducted in accordance with internationally accepted principles for laboratory animal use and care.

**Treatment of Animals**

The rats were divided into two sets of four groups of six animals each; set A for pentobarbital-induced sleeping time study and set B for microsomal drug-metabolizing enzyme study. Groups one to three of each set received reconstituted TN extract 28 mg $kg^{-1}$ (normal human dose), 280 mg $kg^{-1}$ (10x normal human dose) and 560 mg $kg^{-1}$ (20x normal human dose), respectively by oral gavage whilst group four of each set was control and received sterilized distilled water for 6 weeks.

**Pentobarbital-Induced Sleeping Time**

The pentobarbital-induced sleeping time was determined using previously described method. The procedure involved injecting experimental animals with (40 mg $kg^{-1}$ body weight; i.p.) of pentobarbital in normal saline. The time difference between the animals completely losing their reflexes after pentobarbital administration and the time they completely regained their righting reflexes was taken as the pentobarbital-induced sleeping time (Nyarko *et al*., 1999).

**Microsomal Preparation and CYP Isozyme Assays**

Hepatic microsomes of control and TN-treated rats were prepared from tissue homogenate (20% w/v) according to the method of Lake (1987) as modified by Anjum *et al*. (1992) by $CaCl_2$ precipitation of the post-mitochondrial fraction and centrifugation at 27,000x g for 15 min in a high speed Avanti J-E refrigerated centrifuge (Beckman Coulter Inc., USA). The microsomal protein content was determined spectrophotometrically at 595 nm (Photometer 4040, Robert Riche G and Co., Germany) by the method of Bradford (1976) using bovine serum albumin as standard. The activities of NADPH-cytochrome c reductase (Williams and Kamin, 1962), p-nitrophenol hydroxylase (Reinke and Moyer, 1985), aminopyrine N-demethylase (Nash, 1953) 4-nitroanisole O-demethylase (Kato and Jillette, 1965) were determined.

**Statistical Analysis**

Analysis of Variance (ANOVA) was employed in testing the significance of differences between the control and the treatment groups, regarding all the assays used. $p<0.05$ were considered significant. Data were presented as Means±SEM.

## RESULTS

**Effect of Extract on Microsomal Protein and CYP-Mediated Enzymes**

The effect of treatment with varying doses of TN on rat hepatic microsomal protein content and selected CYP-mediated drug-metabolism enzymes are shown in Fig. 1-5. There were insignificant increases ($p>0.05$) in microsomal protein content at all doses of TN (3.25-31%) compared to control, in a non-dose-dependent fashion, with TN at 10x the normal dose (280 mg $kg^{-1}$) causing the highest percentage increase of 31% (Fig. 1). In Fig. 2, rat hepatic microsomal NADPH cytochrome c ($P_{450}$) reductase activity was generally insignificantly reduced ($p>0.05$) in TN-treated animals relative to control (8.9-26.1%). The pNPH activity on the other hand was generally insignificantly ($p>0.05$) elevated (14.8-23%) in the TN-treated rats compared to control (Fig. 3). There were insignificant differences in the activities of rat hepatic microsomal aminopyrine N-demethylase (AmD) and 4-nitroanisole O-demethylase (NOD) between the normal and 10x the normal TN-treated rats relative

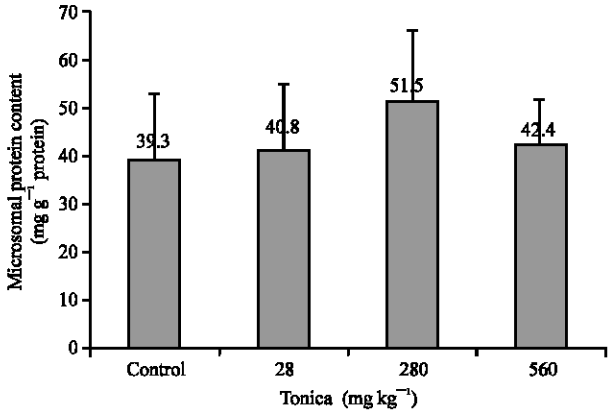


Fig. 1: Effect of Tonica on rat hepatic microsomal protein content. Values are Means±SEM (n = 6). For treatment regimen

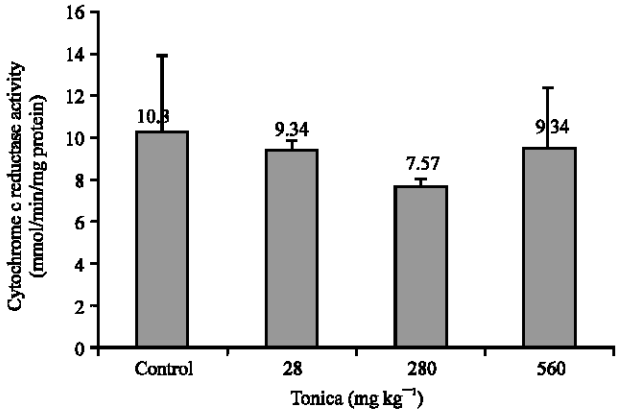


Fig. 2: Effect of Tonica on rat hepatic microsomal cytochrome c (P450) reductase activity. Values are Means±SEM (n = 6) For treatment regimen, see materials and methods

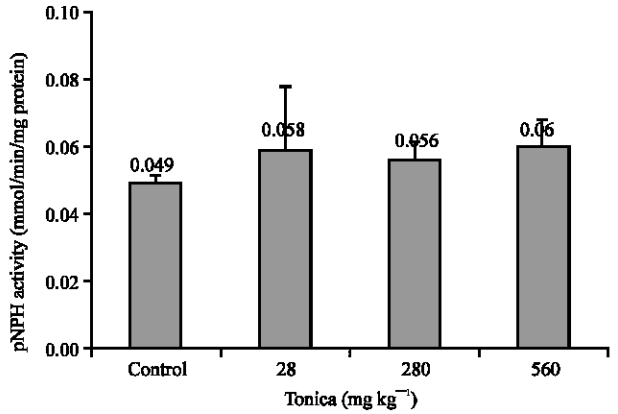


Fig. 3: Effect of Tonica on rat hepatic microsomal p-nitrophenol hydroxylase activity (pNPH) activity. Values are Means±SEM (n = 6)

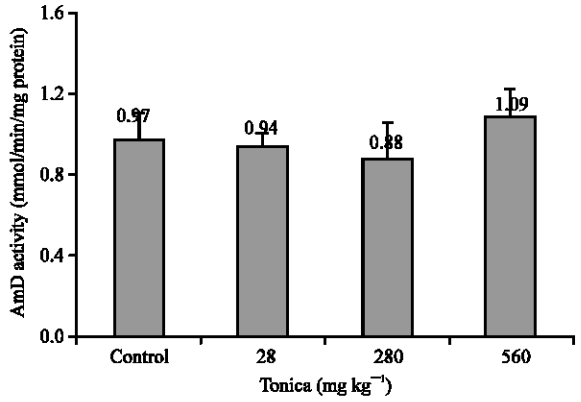


Fig. 4: Effect of Tonica on rat hepatic microsomal aminopyrine N-demethylase (AmD) activity. Values are Means±SEM (n = 6)

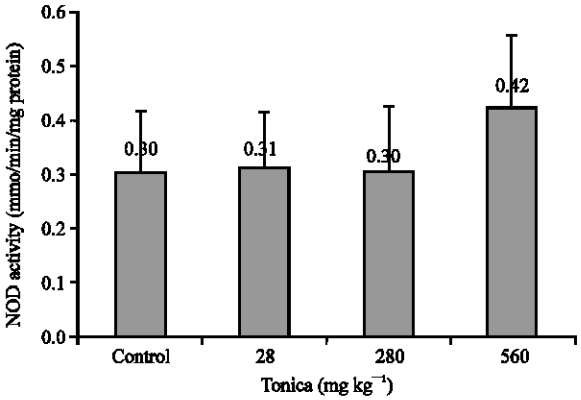


Fig. 5: Effect of Tonica on rat hepatic microsomal nitroanisole O-demethylase (NOD) activity. Values are Means±SEM (n = 6)

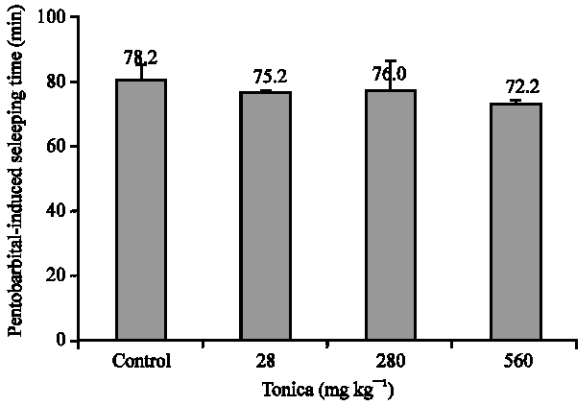


Fig. 6: Effect of Tonica pre-treatment of rats on pentobarbital-induced sleeping time. Values are Means±SEM (n = 6)

to the control. However, at 20x the normal dose of TN (560 mg $kg^{-1}$), AmD and NOD activities were insignificantly ($p>0.05$) elevated 11.7 and 39.8%, respectively above controls (Fig. 4, 5).

**Effect of Extract on Pentobarbital-Induced Sleeping Time**

Figure 6 shows the effect of Tonica pre-treatment of rats on pentobarbital-induced sleeping time. There appears to be insignificant ($p>0.05$) reductions in pentobarbital-induced sleeping time as a result of treatment of animals with varying doses of TN (2.81-7.67%).

## DISCUSSION

Cytochrome $P_{450}$ enzymes (CYPs) are involved in the metabolism of a wide spectrum of foreign and endogenous compounds (Thummel and Wilkinson, 1998). Concurrent consumption of two or more drugs may result in drug-drug interactions, the results of which could have many clinical implications (Zhou *et al*., 2007). Herb-drug or herb-herb interactions result from modulation of CYPs expression or activity, mediated by certain chemicals (Souad *et al*., 2007). Inactivation or inhibition of CYPs may cause severe drug toxicity generally resulting from reduced biotransformation of the drug and thus its accumulation in the body (Sharma and Sangraula, 2002). In contrast, stimulation or induction of CYP may enhance drug metabolism and thus facilitates the clearance of the drug from the body. When this happens the therapeutic potential associated with the drug may not be realised (Bekhti and Pirotte, 1987; Wen *et al*., 1994).

As shown in Fig. 1, there were insignificant increases in the microsomal protein content at all doses of TN relative to the control, in a non-dose-dependent manner with 10x the normal dose causing the highest increase in protein content. These increases may be indicative of induction or stimulation of protein synthesis by TN. The proteins may either be CYP or non-CYP enzyme proteins. Increase in non-enzyme protein may have consequences on specific activities of all CYP-dependent enzymes as seen in the reduction in these activities in the 10x the normal dose TN-treated animals.

In comparison with the control, there was a general insignificant decrease in cytochrome c ($P_{450}$) reductase activity in all the TN-treated groups (Fig. 2), indicating that the electron transport activity was not greatly altered by administration of TN. The marked reduction in enzyme specific activity shown by 10x the normal dose of TN as compared to the other dosage groups may be explained by the marked but insignificant increase in the hepatic microsomal protein content at this dosage (Fig. 1).

The insignificant increases in pNPH activity (14.8-23%) among the treatment groups compared to the control (Fig. 3) are indicative of induction/stimulation of CYP2EI isoform known to mediate pNPH activity (Koop, 1992). Hence care must be taken when other substances that modulate microsomal CYP2EI activity, such as paracetamol, caffeine, chlorzoxazone and enflurane, are co-administered with TN. Twelve percent increase in AmD at 20x the normal dose (Fig. 4) suggests the slight stimulation/induction of CYP2B1 isoform, which is known to modulate AmD activity (Burke *et al.*, 1985). There appears, therefore, to be a threshold dose below which stimulation/induction of AmD may not occur. The marked but insignificant elevation of NOD activity at 20x the normal dose (Fig. 5) is an indication of the induction/stimulation of CYP2A6 or CYP2E1 isoforms, known to modulate NOD activity (Jones *et al*., 1997). It is pertinent to note that the $LD_{50}$ of TN is >5,000 mg $kg^{-1}$, which is about 10x the highest dose of TN used in this study.

One of the ways to study drug interactions is to evaluate the effect of one drug on the metabolism and biological effect of another via the microsomal drug-metabolizing system. In this study, although TN modulated certain CYP isoforms, to some degree, this was not reflected in the metabolism of pentobarbital and its pharmacological effect; modulation of pentobarbital-induced sleeping time (Fig. 6). This suggests that these CYP isoforms may not be involved in the metabolism of pentobarbital but this does not preclude the possibility of these CYP isoforms modulating the metabolism of other

drugs. Herb-drug interactions are known to be caused by phytochemicals which are capable of altering CYP activity (Venkataramanan *et al*., 2006). For example, St. John's wort, an extract of the flowering portion of the plant *Hypericum perforatum* L., containing hyperforin with antidepressant properties, has a strong affinity for Steroid Xenobiotic Receptor (Nathan, 1999). Its binding to the receptor promotes the expression of CYP3A4 gene, thus inducing the enzyme in the liver and intestines resulting in enhanced reduction in the levels of other compounds, whose clearance is mediated by CYP3A4 (Moore *et al*., 2000; Barone *et al*., 2001).

In conclusion, it may be said that TN insignificantly elevated pNPH, AmD and NOD activities to various degrees at the highest dosage of 560 mg $kg^{-1}$, which represents 20x the normal human dose. Thus, although TN at the normal human dose had no effect on these enzyme activities, it is possible that if administered at this dose over a prolonged period and in the presence of reduced renal clearance, may result in high plasma levels similar to or above that caused by the highest dose used in this study. This may cause the induction of CYP2E1, CYP2B1 and CYP2A6 isoforms with implications for drug-drug interactions. Therefore, care must be taken in the co-joint administration of TN with other herbal products or substances like paracetamol, caffeine, chlorzoxazone and enflurane.

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