

## THE EFFECT OF ST JOHN'S WORT (*HYPERICUM PERFORATUM*) ON CYTOCHROME P450 1A2 ACTIVITY IN PERFUSED RAT LIVER

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**Aims.** We previously reported the effect of St John's wort (*Hypericum perforatum*) standardised extract LI 160 on the activities of cytochrome P450 2C6, 2D2 and 3As (Dostalek et al., Life Sciences 2005;78(3):239-44). In this study, we aimed to assess the effect of St John's wort on the activity of cytochrome P450 1A2.

**Methods.** The isolated perfused rat liver model was used in our experiments with phenacetin as a marker substrate for cytochrome P450 1A2. Male Wistar rats were treated with St John's wort extract (100 mg/kg, once daily for 10 days); comparative inhibitor (alpha-naphthoflavone, 100 mg/kg) or comparative inducer (omeprazole, 30 mg/kg).

**Results.** The rate of formation of acetaminophen was significantly inhibited by the use of St John's wort (P<0.001). In addition, St John's wort extract inhibited cytochrome P450 1A2 activity significantly more than the control inhibitor (P<0.001).

**Conclusions.** St John's wort significantly inhibited cytochrome P450 1A2 enzyme activity. It remains to be determined whether the co-administration of St John's wort extract and other medications or substrates for cytochrome P450 1A2 could result in significant drug interactions.

### INTRODUCTION

The market and interest for herbal medicines and food supplements in the Western world has markedly increased in recent years, and, not surprisingly, reports of its interactions with conventional drugs have increased. The most well-known and documented example is the interaction of St John's wort with a variety of concomitantly used drugs<sup>1</sup>. Many case reports showing interactions between St John's wort and anticonvulsants, anti-HIV drugs, antidepressants, immunosuppressant agents and oral contraceptives have been published. Based on clinical and experimental data, the co-administration of St John's wort should be avoided particularly in patients who are taking drugs with a narrow therapeutic range<sup>2</sup>.

Although St John's wort (*Hypericum perforatum*) extracts are among the best characterized herbal medicines, numerous studies have reported the inductive and inhibitory effects of St John's wort on cytochrome P450 activity<sup>1,3-5</sup>. Many clinical trials have shown that St John's wort induces P450 2C19, 2E1 and 3A4 with no effect on P450 2C9 (ref.<sup>2</sup>). However, the literature is very contradictory concerning the effect of St John's wort on P450 1A2 enzyme activity. A single study associated treatment with St John's wort with a decreased plasma concentration of theophylline (a marker of P450 1A2) (ref.<sup>6</sup>), and this was later confirmed<sup>1,7</sup>. Further, an increased protein level of P450 1A2 after extended treatment with St John's wort has been reported by Shibayama et al.<sup>8</sup> On the other

hand, Wenk et al.<sup>9</sup> and Arold et al.<sup>10</sup> reported no change in P450 1A2 activity. In addition, *in vitro* data reported by Obach demonstrated the inhibitory effect of St John's wort constituents on P450 1A2 activity<sup>11</sup>.

There are many different brands of St John's wort available at the market, and as with any crude extract of a natural product, the content of its active ingredients varies considerably between manufacturers and between batches from the same manufacturer. The aim of our study was to describe the effect of commercially available *Hypericum perforatum* standardized extract LI 160 (containing 280 µg of hypericin, 300 µg of pseudohypericin, 6.5 mg of hyperforin and 10 mg of hyperosid in a single dose of 100 mg/kg) on the activity of P450 1A2. In order to characterize this effect, isolated perfused rat liver was chosen as the study model.

### MATERIALS AND METHODS

#### Chemicals

The standardized extract LI 160 was kindly provided by Lichtwer Pharma AG (Berlin, Germany); phenacetin, acetaminophen, alpha-naphthoflavone and omeprazole were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals and solvents used for HPLC assays were of HPLC or analytical grade and were obtained from Merck (Darmstadt, Germany) or Fluka Chemie AG (Buchs, Switzerland). All chemicals were used without

further purification. All water solutions were prepared with Ultrapure water (Premier MFG Systems, Phoenix, AZ), filtered through a 0.45 µm nylon membrane filter.

#### Animals

The experiment was carried out on male Wistar rats (weighing 220 ± 25 g, BioTest, Konarovice, Czech Republic) with free access to food and water under controlled environmental conditions (lights on from 6:00 am to 6:00 pm, temperature 21-22 °C, relative humidity 50-60%). After at least 10 days of adaptation to standard laboratory conditions, the rats were randomly allocated to 4 groups and treated intraperitoneally according to the following design: a) control group (n = 8) – sterile saline (2 ml/kg), once daily for 10 days, the last dose was applied 24 h before liver preparation; b) St John's wort group (n = 10) – standardized extract LI 160 (100 mg/kg) once daily for 10 days, the last dose was applied 24 h before liver preparation; c) comparative inhibitor group (n = 8) – sterile saline once daily for 10 days followed by a single dose of alpha-naphthoflavone used as a control inhibitor of P450 1A2 (100 mg/kg); d) comparative inducer group (n = 8) – sterile saline once daily for 5 days followed by five doses of omeprazole, a control inducer of P450 1A2 (30 mg/kg). The doses of control substrates were chosen because these were effective in a pilot study performed in our laboratory.

#### Liver perfusion

The isolated perfused rat liver model was used in our experiments. All experimental procedures were approved by the by the Czech Central Commission for

Animal Welfare according to Czech Act No. 246/1992 Coll. Aliquots of liver perfusate were collected at the 30<sup>th</sup>, 60<sup>th</sup> and 120<sup>th</sup> min of perfusion and stored at -30 °C until analysis. All methods used in the study have been described elsewhere<sup>12</sup>. Liver viability was monitored in terms of constant perfusion pressure and rate, pH of perfusate, oxygen consumption, liver weight and a visual examination after finishing the perfusion.

#### Chromatographic conditions

Phenacetin (12.5 mg/l) was used as a specific marker of P450 1A2 activity. The HPLC determination of phenacetin and its respective metabolite was determined by a previously published and validated method<sup>13</sup>. Briefly, a mixture of 500 µl of liver perfusate and 50 µl of internal standard solution (40 µg/ml of chlorpropamide) and 4 ml of diethyl ether was vortexed (10 min at 2 000 rpm) and then centrifuged (5 min at 3 000 × g). The organic phase was decanted and evaporated under a nitrogen stream, the residue was redissolved in 150 µl of mobile phase (10 mM monobasic potassium phosphate, pH 4.5 and acetonitrile, 65:35, v/v). The analytes were separated in a Luna C18(2) analytical column (150 × 4.6 mm, 5 µm) from Phenomenex (Torrance, CA) at 40 °C. Absorbance was monitored at 245 nm. A flow rate gradient was used for the separation: 1.0 ml/min for 0-3 min, followed by 1.8 ml/min for 3-10 min.

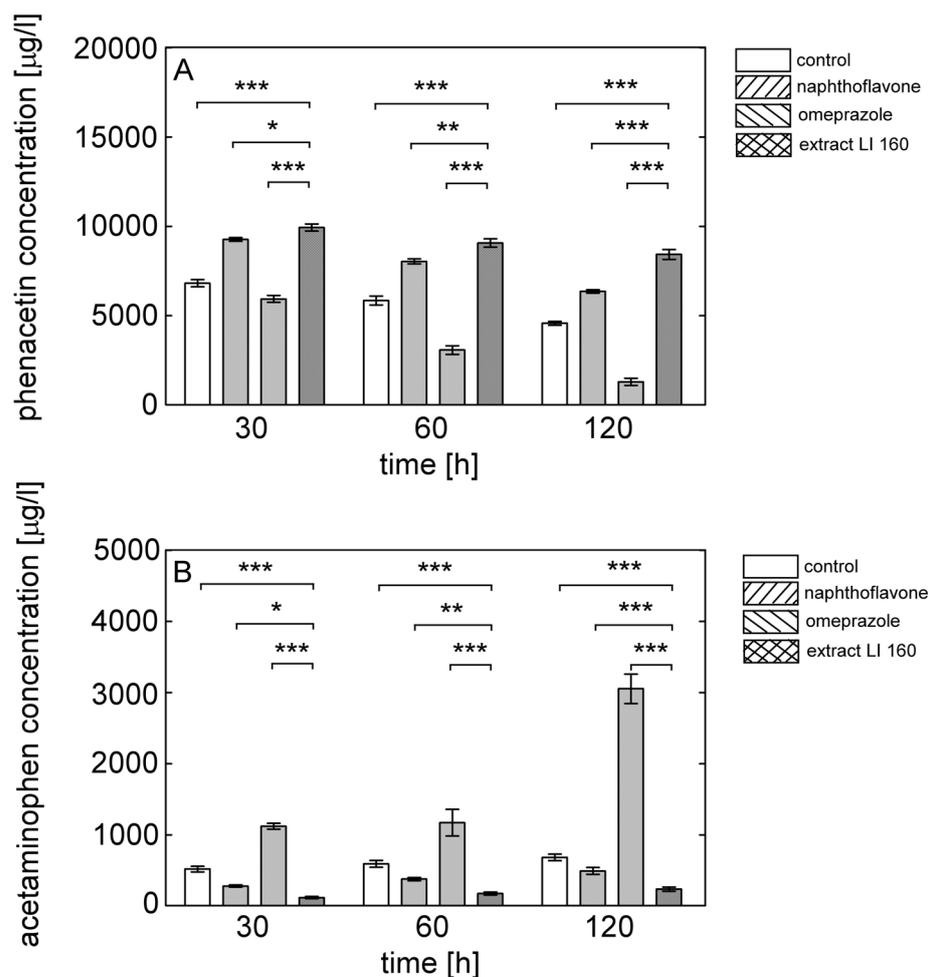
#### Statistical analysis

Phenacetin and acetaminophen perfusate concentrations vs. time data were analysed according to a non-compartment pharmacokinetic model. The data were

**Table 1.** Pharmacokinetic parameters of phenacetin and acetaminophen in the isolated perfused rat liver after administration of sterile saline (2 ml/kg, i.p.), once per day for 10 days (control group); standardized extract LI 160 (100 mg/kg, i.p.) once per day for 10 days (St John's wort group); sterile saline once per day for 10 days followed by a single dose of alpha-naphthoflavone (100 mg/kg, i.p.) (comparative inhibitor group); and sterile saline once per day for 5 days followed by five doses omeprazole (30 mg/kg, i.p.) (comparative inducer group).

Group	Number	C <sub>max</sub> acetaminophen (µg/l)	t <sub>1/2</sub> phenacetin (h)	Cl phenacetin (l/h)	AUC phenacetin (µg l/h)	AUC acetaminophen (µg l/h)
Control	8	682 ± 4.5	2.4 ± 0.13	0.34 ± 0.01	12 570 ± 294	639 ± 21
α-Naphthoflavone	8	490 ± 48***	3.3 ± 0.1***	0.19 ± 0.008***	16 321 ± 241***	466 ± 31***
Omeprazole	8	3 050 ± 208***	0.7 ± 0.09 ***	1.12 ± 0.04***	9 102 ± 368 ***	1 092 ± 56 ***
Extract LI 160	10	237 ± 29***	6.1 ± 0.2 ***	0.06 ± 0.002***	19 654 ± 249 ***	303 ± 19 ***

Data are expressed as means ± SEM and statistical significance related to a control group. \*\*\*, P < 0.001. C<sub>max</sub> acetaminophen – maximum acetaminophen perfusate concentration, t<sub>1/2</sub> phenacetin – perfusate elimination half life of phenacetin, Cl phenacetin – clearance of phenacetin, AUC phenacetin – area under the entire perfusate concentration curve of phenacetin, AUC acetaminophen – area under the entire perfusate concentration curve of acetaminophen.



**Fig. 1.** Concentration of phenacetin (A) and acetaminophen (B) in perfusate medium from isolated perfused rat liver experiment after administration of sterile saline (2 ml/kg, i.p.), once per day for 10 days (control group); standardized extract LI 160 (100 mg/kg, i.p.) once per day for 10 days (St John's wort group); sterile saline once per day for 10 days followed by a single dose of alpha-naphthoflavone (100 mg/kg, i.p.) (comparative inhibitor group); and sterile saline once per day for 5 days followed by five doses of omeprazole (30 mg/kg, i.p.) (comparative inducer group). Data are expressed as means  $\pm$  SEM and statistical significance related to a control group. \*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ .

analysed by Kinetica version 4.1 (InnaPhase Corporation, Philadelphia, PA). The statistical analysis was carried out with SPSS software (version 16, Chicago, IL). Data are expressed as means  $\pm$  SEM. Values of  $P < 0.05$  were considered to be significant.

## RESULTS AND DISCUSSION

Standardized extract LI 160 was well-tolerated by all the animals, the body and liver weights of rats treated with St John's wort extract ( $246.0 \pm 20.0$  g and  $10.5 \pm 1.5$  g, respectively) were similar to those in control rats ( $250.0 \pm 15.0$  g and  $10.0 \pm 2.0$  g, respectively).

### Effect of St John's wort on P450 1A2 activity

The effect of St John's wort extract LI 160 on the pharmacokinetics of phenacetin is demonstrated in (Fig. 1) and (Tab. 1). St John's wort extract LI 160 inhibited

phenacetin deethylation via P450 1A2, which resulted in a significant increase in the AUC of phenacetin ( $P < 0.001$ ) and  $t_{1/2}$  of phenacetin ( $P < 0.001$ ). On the other hand, the use of extract LI 160 resulted in a significant decrease in the Cl of phenacetin ( $P < 0.001$ ) and AUC of acetaminophen ( $P < 0.001$ ). St John's wort extract LI 160 inhibited the P450 1A2 metabolic activity significantly more than the control inhibitor, alpha-naphthoflavone.

We previously reported that St John's wort resulted in a significant increase in P450 3As enzyme activity and to a significant inhibition of P450 2C6 activity<sup>12</sup>. In this experiment, St John's wort extract LI 160 was shown to powerfully inhibit P450 1A2.

Although St John's wort extracts are probably the best characterized herbal medicines, more than 150 ingredients, or groups of ingredients, have been identified so far<sup>14</sup>. Still, 30-50% of its compounds are not yet structurally defined; some of these might well contribute to the drug-drug interactions<sup>14</sup>. Flavonoids are widespread

compounds found in the plant kingdom. The total flavonoid content is dependent on such factors as growth environment, the quantities of various plant parts used, harvest time, drying procedures, and storage conditions<sup>15</sup>. St John's wort extract contains approximately 20 kinds of flavonoids some of which (hyperoside, isoquercitrin, quercitrin, quercetin) inhibit drug metabolizing enzymes (especially P450 1A and 2C) in human liver<sup>16</sup>. The flavonoid I3,II8-biapigenin has been shown to be a P450 1A2 inhibitor with  $K_i = 0.95 \pm 0.22 \mu\text{M}$  (ref.<sup>11</sup>). A less potent inhibitory effect on P450 1A2 enzyme activity has been found for naphthodianthrone derivatives (hypericin,  $K_i > 100 \mu\text{M}$ ), phloroglucinol derivatives (hyperforin,  $K_i > 100 \mu\text{M}$ ) and phenolic acids (chlorogenic acid,  $K_i > 100 \mu\text{M}$ ) (ref.<sup>11</sup>).

A drug interaction occurring *in vivo* is likely if the maximum plasma unbound concentration /  $K_i > 0.2$  (ref.<sup>17</sup>). The maximum plasma concentration ( $C_{\text{max}}$ ) of hyperforin is 0.17-0.50  $\mu\text{M}$  in humans following a single dose (300 mg) of standardized St John's wort extracts containing 5% hyperforin<sup>18,19</sup>. Thus, the less potent inhibitor could contribute more to the inhibition of P450 than the most potent inhibitor present in a minor amount. However, with a complex mixture of compounds, each compound in the preparation (depending on its relative abundance) could have an impact on drug-drug interactions.

The importance of uridine 5'-diphosphate-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15 in the biotransformation of acetaminophen has been demonstrated<sup>20,23</sup>. However, to the best of our knowledge, the effect of St John's wort extracts has not been studied. Thus, further studies are required to explain the effect of St John's wort extracts on the activity of phase II enzymes involved in the biotransformation of acetaminophen as their induction can influence the level of acetaminophen.

## CONCLUSIONS

In conclusion, the standardized extract LI 160, a herbal preparation used in the treatment of depression, has been identified as a potent inhibitor of the metabolic activity of P450 1A2. However, it remains to be determined whether the co-administration of St John's wort and other medications could result in significant drug interactions via the inhibition of P450 1A2 activities.

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