Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 2, 2011 ISSN - 0974-2441

Research Article

HERB-DRUG INTERACTION STUDIES OF *EURYCOMA LONGIFOLIA* EXTRACT TAF-273 ON THE METABOLISM OF ROSIGLITAZONE, AN ANTIDIABETIC DRUG

PURWANTININGSIH[1,3], ABAS HJ HUSSIN[1,2*], KIT LAM CHAN[1]

[1]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia, [2]Centre for Drug Research, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia, [3]Department of Pharmacology & Clinical Pharmacy, Faculty of Pharmacy, Gadjah Mada University, 55281 Yogyakarta, Indonesia.
Email: abas_hussin@yahoo.com

ABSTRACT

Presently, many natural products recommended for treatment of erectile-dysfunction are easily available in the market and one of them is *Eurycoma longifolia*. The belief that natural products are safer than synthetic drugs has led to the dramatic growth of herbal medicine usage. However, like synthetic drugs, these preparations may also cause adverse effects and drug interactions, therefore may affect the therapeutic effect of allopathic medicine. The present study evaluated a standardized extract TAF-273 of *Eurycoma longifolia* on the metabolism of an antidiabetic drug, rosiglitazone. Male Sprague-Dawley (SD) rats were divided into two main groups: normal and diabetic-induced. Each group was further divided into three sub-groups; old, adult and young. By using the collagenase perfusion technique, isolated hepatocytes were prepared from these rats, a total of six rats (n=6) per group and rosiglitazone N-demethylase assay was then determined by measuring the quantity of formaldehyde formed by using a microplate reader at 415 nm. A significant increase in formaldehyde concentration was observed in the old, adult normal rats and the old diabetic rats ($p < 0.05$) but there was no significant difference in adult and young diabetic male rats ($p>0.05$) when compared to the control group, respectively. In the young, normal male group, a significant increase was only found at the highest concentration of TAF-273 tested (100 µg/mL). In general, TAF-273 displayed a higher increase in formaldehyde concentration in the old group for both normal and diabetic male rats. A significant increase in formaldehyde concentration was observed on TAF-273 treatment in old normal and diabetic male rats ($p<0.05$), suggesting that TAF-273 increased the phase 1 rosiglitazone metabolism in male rat hepatocytes.

Keywords: Standardized extract of *Eurycoma longifolia* Jack (Simaroubaceae), N-demethylase activity, rosiglitazone, rat hepatocytes.

INTRODUCTION

All over the world, there are many kinds of remedies based on herbal medicine[1]. Various traditional medicines use isolated compound forms or preparations consisting of whole extracts, purified extracts or a mixture of identified compounds from natural sources, such as *Eurycoma Longifolia* extracts[2,3], to cure erectile-dysfunction (ED).

Eurycoma longifolia Jack *(E. longifolia)* is one of the traditional medicines that is widely used in Asia especially in Indonesia, Malaysia and Vietnam[4]. At present, more than 200 *E. longifolia* products are circulated in the Malaysian market and many of these products focus on the aphrodisiac qualities[5].

E. longifolia roots have many constituents such as alkaloids[6,7], quassinoids[8,9], squalene derivatives[10], biphenylneolignan[11] and tirucallane-type triterpenes[12]. Studies on *E. longifolia* that have been done include antiplasmodial activity[13,14], plant growth inhibitors, anti-tumor promoting, anti-schistosomal and anti-parasitic activity[15,16], cytotoxic effect[17], antypiretic activity[18], apoptosis effects in HepG2 cells[19] and aphrodisiac property[20-22]. However, to date no study has been published pertaining its herb-drug interaction.

Rosiglitazone is a potent antihyperglycemic agent[23,24] that reduces insulin resistance in patients with type 2 diabetes[25]. In humans, the major routes of metabolism are *N*-demethylation and hydroxylation with subsequent conjugation. Glucuronide conjugation is found to be lower (7.5 percent when administered) than sulphate conjugation (37.8 percent)[26,27]. Diabetes mellitus, cardiovascular diseases and hypogonadism have been associated significantly with sexual dysfunction[28]. According to a new study[29-31], testosterone deficiency, previously recognized as common in men with type 2 diabetes, is also common in men with type 1 diabetes. Low testosterone levels may cause health problems that can lead to erectile dysfunction[32].

It is possible for diabetic patients who experience erectile dysfunction to use *E. longifolia* and rosiglitazone concurrently. Both *E. longifolia* and rosiglitazone have to be consumed regularly over time so interactions may occur that might affect the drug therapeutic effect. The present study examined the effect of the standardized extract of *E. longifolia* (TAF-273) on rosiglitazone metabolism, especially on N-demethylase activity.

MATERIALS AND METHODS

Plant material

TAF-273 extract was made from the *E. longifolia* roots according to the method previously described[13] and was standardized in the Laboratory of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Universiti Sains Malaysia. From the extract, pure eurycomanone had been isolated and characterized, it contains 19.6% of eurycomanone[33]. Standardized eurycomanone-enriched extract of *E. longifolia* (TAF-273) was used in this study and was prepared by dissolving the extract in the distilled water. A voucher specimen of the plant was deposited, with Reference No. 785-117, at the Penang Botanical Garden[13].

Chemicals

Rosiglitazone was purchased from Wuhan Sunrise Technlogy, China. Diethyl ether, magnesium chloride and magnesium sulphate were purchased from BDH Laboratory Supplies, UK. Acetyl acetone, streptozotocin, collagenase type IV and trypan blue were obtained from Sigma Chemicals (St. Louis, MO, USA). Disodium hydrogen phosphate, ammonium acetate, barium hydroxide, zinc sulphate heptahydrate were purchased from R & M Chemicals, UK, while glucose monohydrate and calcium chloride from Riedel-deHaen, France. Formaldehyde solution 37 percent was obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical grade.

Experimental animals

The rats for this experiment were bred in the animal house at Universiti Sains Malaysia, Penang, Malaysia. Male and female *Sprague-Dawley* (SD) rats with different ages were used (young = 6-8 weeks, adult = 12-16 weeks and old 20-24 weeks) and caged according to their body weight (young males and females = 120±20 and 100±20 g; adult males and females = 200±30 and 170±20 g; old males and females= 350±40 and 250±50 g). All the rats had access to standard food pellets (Gold Coin®, Penang, Malaysia) and tap water *ad libitum.* The study protocol was approved by the Animal Ethics Committee, University Sains Malaysia, Penang, Malaysia with approval no. USM/PPSF/50 (066) Jld.2.

Induction of animal diabetes mellitus by streptozotocin (STZ)

Diabetes mellitus in the SD rats was induced by using 60 mg/kg b.w. of streptozotocin (STZ) intravenously. The STZ (prepared fresh and dissolved in normal saline 0.9 percent) was injected immediately into the rat's tail vein under ether anesthesia. The blood glucose level in the rats was measured after three days of the STZ injection. The SD rats were considered as diabetic if the glucose level was higher than 15.6 mmol/L at fasting state.

Effect of TAF-273 on antidiabetic drug (rosiglitazone) metabolism

Phase 1 liver metabolizing enzymes may be affected by certain biological factors such as age, gender and disease. Therefore, two main groups of SD rats: normal and diabetic, were used in this study. Then, each group was divided into three sub-groups: old, adult and young females and males (n=6). Rat hepatocytes were isolated by using the collagenase perfusion technique from these groups[34]. The viability of the hepatocytes was determined by using trypan blue and only hepatocyte samples with the percentage of cell viability above 85 percent were used.

Equal volumes (1.0 mL) of the serial dilutions of TAF-273 (0.001 to 100 µg/mL) in distilled water were added into petri dishes containing incubation medium, freshly isolated hepatocytes (75,000 cells) and rosiglitazone (0.75 mM). For the control test dishes, the herbal preparation was replaced with distilled water. The petri dishes were incubated at room temperature for ten minutes on a table top shaker. $ZnSO_4$ [0.5 mL; 25 percent (w/v)] was added to terminate the reaction and this was followed by the addition of saturated $Ba(OH)_2$ (0.5 mL).

The samples were then centrifuged for ten minutes at 3000 rpm. 1 mL of supernatant was added to 2 mL of Nash reagent and incubated at 60 ºC for 30 minutes in a waterbath shaker. Rosiglitazone N-demethylase activity was determined by measuring the quantity of formaldehyde formed at 415 nm by using a microplate reader according to the colorimetric method of Nash (1953)[35].

Data analysis

The means and standard deviations of the formaldehyde formed (µg/mL) due to the N-demethylase activity in the treatment groups were calculated and compared with the control group. The percentage of formaldehyde formed (PFF) was calculated by dividing formaldehyde formed in the test groups with formaldehyde formed in control group, respectively.

The results were analyzed using the ANOVA and the Tukey Test and the level of significance was set at $p<0.05$. The effect of age, gender, disease and TAF-273 concentrations on the formaldehyde formed were evaluated by using a univariate analysis.

RESULTS AND DISCUSSION

The effect of TAF-273 on phase 1 rosiglitazone metabolism in normal and diabetic male rat hepatocytes is shown in Table **1**. In general, there were some elevations on formaldehyde formed however, the increase were not all significantly different.

A significant effect occurred in old, adult, normal male rats and old, diabetic male rats ($p<0.05$) but there was no significant difference in adult and young diabetic male rats ($p>0.05$) when compared to the control group respectively. In the young, normal, male group, a significant effect was only found in the highest concentrations of TAF-273 tested (100 µg/mL). TAF-273 showed a significant effect ($p<0.05$) on N-demethylase activity in almost all the used concentrations (0.001 to 100 µg/mL) in old, normal and diabetic rat hepatocytes.

A significant elevation was found in the adult, normal group rats in the presence of TAF-273 at concentrations of 1, 10 and 100 µg/mL as compared with the control rosiglitazone. In general, TAF-273 displayed a higher effect in the old group for both normal and diabetic males (Figs. 1 and 2).

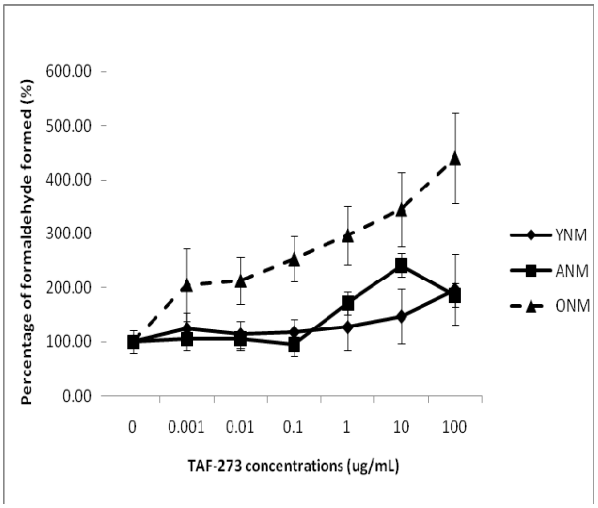

Fig. 1: The percentage of formaldehyde formed (%) in young [YNM], adult [ANM] and old [ONM] normal male rat hepatocytes treated with TAF-273 at concentrations of 0.001 to 100 µg/mL as compared to their respective control group (n=6)

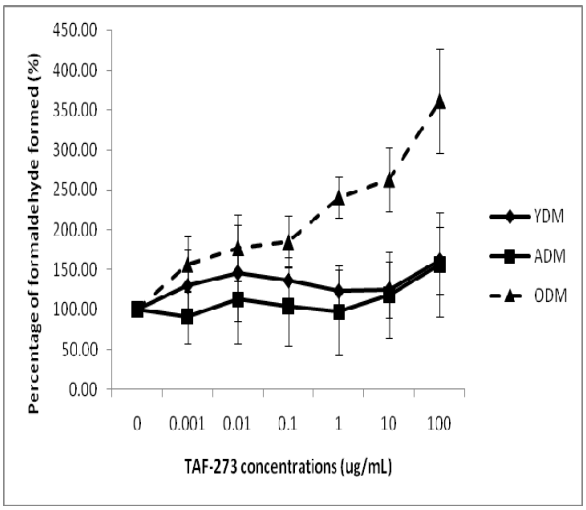

Fig. 2: The percentage of formaldehyde formed (%) in young [YDM], adult [ADM] and old [ODM] diabetic male rat hepatocytes treated with TAF-273 at concentrations of 0.001 to 100 µg/mL as compared to their respective control group (n=6)

The statistical test results of the TAF-273 effect on phase 1 hepatic drug metabolizing enzymes in normal and diabetic female rat hepatocytes are showed in Table **2**. There were significant changes in all the normal, female groups.

However, in the diabetic female groups, a significant increase in N-demethylase activity was observed in the adult group in the presence of 100 µg/mL ($p<0.05$) of TAF-273. Old and young female SD rats showed no significant difference in N-demethylase activity after being treated with 0.001 to 100 µg/mL of TAF-273 when compared to the control group.

The percentage increase of the formaldehyde formed, after being treated with 0.001 to 100 µg/mL of TAF-273, was similar in young, adult and old groups for normal and diabetic female rats (Figures 3 and 4).

Table 1: Effect of TAF 273 on rosiglitazone metabolism in male SD rat hepatocytes

Concentrations of *E. longifolia* extract (µg/mL)	Concentrations of formaldehyde formed (µg/mL) (Mean ± SD)					
	Old Normal Male Rat	Adult Normal Male Rat	Young Normal Male Rat	Old Diabetic Male Rat	Adult Diabetic Male Rat	Young Diabetic Male Rat
100	1.027 ± 0.195***	0.404 ± 0.115**	0.386 ± 0.127**	0.853 ± 0.155***	0.629 ± 0.262	0.394 ± 0.102
10	0.805 ± 0.161***	0.525 ± 0.149***	0.290 ± 0.099	0.621 ± 0.094***	0.476 ± 0.214	0.306 ± 0.087
1	0.693 ± 0.127***	0.372 ± 0.071*	0.249 ± 0.085	0.567 ± 0.060***	0.388 ± 0.215	0.300 ± 0.078
0.1	0.591 ± 0.098**	0.207 ± 0.020	0.231 ± 0.048	0.436 ± 0.077*	0.418 ± 0.199	0.332 ± 0.120
0.01	0.498 ± 0.101*	0.231 ± 0.038	0.223 ± 0.050	0.418 ± 0.099*	0.452 ± 0.223	0.356 ± 0.148
0.001	0.479 ± 0.158*	0.228 ± 0.051	0.244 ± 0.058	0.370 ± 0.082	0.364 ± 0.133	0.316 ± 0.113
Control	0.233 ± 0.028	0.217 ± 0.013	0.196 ± 0.008	0.236 ± 0.045	0.402 ± 0.179	0.244 ± 0.056

Note: * < 0.05 (significantly different as compared to control group),** < 0.01 (significantly different as compared to control group), *** <0.001 (significantly different as compared to control group

Table 2. Effect of TAF 273 on rosiglitazone metabolism in female SD rat hepatocytes

Concentrations of *E. longifolia* extract (µg/mL)	Concentrations of formaldehyde formed (µg/mL) (Mean ± SD)					
	Old Normal Female Rat	Adult Normal Female Rat	Young Normal Female Rat	Old Diabetic Female Rat	Adult Diabetic Female Rat	Young Diabetic Female Rat
100	0.549 ± 0.201*	0.538 ± 0.124***	0.511 ± 0.095***	0.498 ± 0.166	0.388 ± 0.098**	0.442 ± 0.099
10	0.439 ± 0.114	0.412 ± 0.126*	0.402 ± 0.080**	0.303 ± 0.095	0.316 ± 0.073	0.370 ± 0.120
1	0.380 ± 0.149	0.356 ± 0.045	0.380 ± 0.068*	0.332 ± 0.118	0.274 ± 0.021	0.332 ± 0.052
0.1	0.359 ± 0.154	0.399 ± 0.070*	0.346 ± 0.057	0.319 ± 0.078	0.279 ± 0.033	0.319 ± 0.070
0.01	0.367 ± 0.158	0.340 ± 0.041	0.335 ± 0.074	0.407 ± 0.162	0.276 ± 0.041	0.338 ± 0.108
0.001	0.287 ± 0.077	0.370 ± 0.108	0.319 ± 0.063	0.388 ± 0.159	0.282 ± 0.041	0.335 ± 0.067
Control	0.252 ± 0.083	0.231 ± 0.013	0.249 ± 0.013	0.308 ± 0.085	0.263 ± 0.045	0.290 ± 0.064

Note: * < 0.05 (significantly different as compared to control group),** < 0.01 (significantly different as compared to control group),*** <0.001 (significantly different as compared to control group)

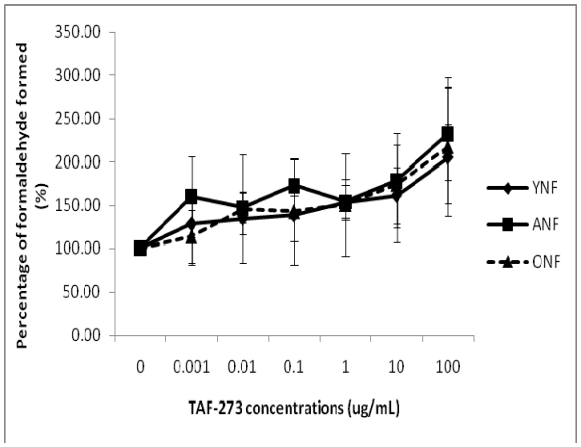

Fig. 3: The percentage of formaldehyde formed (%) in young [YNF], adult [ANF] and old [ONF] normal female rat hepatocytes treated with TAF-273 at concentrations of 0.001 to 100 µg/mL as compared to their respective control group (n=6)

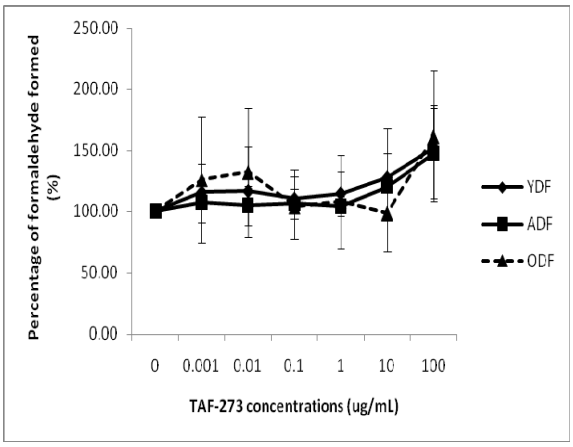

Fig. 4: The percentage of formaldehyde formed (%) in young [YDF], adult [ADF] and old [ODF] diabetic female rat hepatocytes treated with TAF-273 at concentrations of 0.001 to 100 µg/mL as compared to their respective control group (n=6)

Based on the univariate test, it can be concluded that there was a significant effect of age, gender and extract concentrations on the formaldehyde formed but this was not affected significantly by diseases. This means that age, gender or extract concentrations can affect rosiglitazone metabolism.

Sex differences in the rate of drug metabolism in rats have been well documented[36,37]. The sensitivity of hepatic metabolizing enzymes to drugs in adults may vary with age and gender[38]. Based on the results, it can be seen that diseases had a significant effect in all the normal groups for both males and females and in just one group for the diabetic group (old, diabetic male group and adult, diabetic female group).

The effect of TAF-273 on phase 1 hepatic rosiglitazone metabolism was observed in male and female rat hepatocytes. It is interesting to note that the observed effects especially in the old, normal and diabetic male groups were significantly different in almost all of the TAF-273 concentrations tested.

Many chronic diseases increase with the prevalence of age and can have an impact on sexual function. According to a new study, testosterone deficiency is commonly recognized in men with type 2 or type 1 diabetes. These findings suggest that there is a direct link between insulin resistance and reduced testosterone levels in men. Approximately 25 percent of men over the age of 65 years have some degree of erectile dysfunction (ED). According to a prominent study on ED prevalence, the Massachusetts Male Aging Study, 52 percent of men aged 40 to 70 years suffer from ED[39]. ED is also known to be a common sexual problem among the aging males of Asia in Japan, Malaysia, Taiwan, China and Korea[2].

E. longifolia is an alternative medicine that has been widely used by society. It is claimed as aphrodisiac and has the effect of increasing testosterone levels. *E. longifolia* does not act in the same way as other claimed aphrodisiacs which take effect immediately.

It has to be consumed regularly over time. Optimal effectiveness should be felt within a week or more of continuous uninterrupted use. It is possible for a diabetic person who experiences sexual dysfunction to use *E. longifolia* concurrently with an antidiabetic agent such as rosiglitazone.

Antidiabetic agents are commonly used for a long period. Rosiglitazone is one of the antidiabetic drugs from the thiazolidinedione group that has a good prospect because it is less toxic as compared to troglitazone or pioglitazone[40,41]. While rosiglitazone is the ligand for PPAR-γ, PPAR-γ is a critical transcription factor that influences numerous genes related to lipid metabolism and glucose homeostasis[42]. If *E. longifolia* and rosiglitazone are used concurrently for a long period, they may affect the therapeutic effects of rosiglitazone.

Based on the observation that TAF-273 increased rosiglitazone metabolism *in-vitro* especially in the old, normal and diabetic male rat groups, some *ex-vivo* or *in-vivo* experiments are needed so that the effect of TAF-273 on rosiglitazone metabolism can be known. On the other hand, the significant effects of TAF-273 in both the normal and diabetic groups probably indicate that TAF-273 can affect not only drug metabolism in diabetic patients, but in normal patients as well. In conclusion, a significant effect on rosiglitazone metabolism by TAF-273 was observed in both the normal and diabetic male and female groups ($p<0.05$) especially in the old, normal and diabetic male groups.

ACKNOWLEDGEMENT

The authors wish to acknowledge Grant Number 1001/PFARMASI/821127, School of Pharmaceutical Sciences, Universiti Sains Malaysia, which was used to support this research project.

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