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Cardio-protective role of *Terminalia arjuna* bark extract is possibly mediated through alterations in thyroid hormones

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Received November 3, 2004, accepted January 20, 2005

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Pharmazie 61: 793–795 (2006)

Terminalia arjuna bark extract is believed to exhibit cardio-protective effects. In the present study we investigated the possible involvement of thyroid hormones in the amelioration of cardiac and hepatic lipid peroxidation (LPO) by a bark extract of the plant in albino rats. While L-thyroxine (L-T_4) treatment increased the level of thyroid hormones, heart/body weight ratio as well as cardiac and hepatic lipid peroxidation, simultaneous administration of 21.42 and 42.84 mg/kg of the plant extract decreased the level of thyroid hormones and also the cardiac LPO, suggesting the possible mediation of the drug action through an inhibition in thyroid function. These effects were comparable to a standard antithyroid drug, propyl thiouracil (PTU). When the drug was administered to euthyroid animals, serum concentrations of thyroid hormones were decreased, whereas the hepatic LPO increased indicating a drug induced toxicity in euthyroid subjects. Although a suboptimal dose of the drug was found to be non-toxic to the liver, it appeared to be of no use, as it could neither affect the thyroid functions nor the cardiac lipid peroxidation. Since in euthyroid animals, thyroid hormones were decreased and hepatic LPO was increased, it is suggested that high amounts of this plant extract should not be consumed, as hepatotoxicity as well as hypothyroidism may be caused.

1. Introduction

Some plant extracts are known to ameliorate cardiovascular problems (Van and Dall 1983; Gupta et al. 2001; Bharani et al. 2002; Nammi et al. 2003). *Terminalia arjuna* bark extract is one of those agents that regulate several cardiovascular disorders including hypertension, hypercholesterolaemia, and angina (Gupta et al. 2001; Bharani et al. 2002; Nammi et al. 2003). However, its mode of action is not yet clear. As thyroid hormones are also involved in cardiovascular problems (Hak et al. 2000; Donatelli et al. 2003; Watanabe et al. 2003; Wald et al. 2003; De Tommasi et al. 2003), it was thought that the cardio protective effect of this drug is possibly mediated through alteration in thyroid hormone concentrations. Therefore, in the present investigation an attempt has been made to explore this possibility in thyroxine induced hyperthyroid rats. Simultaneously toxicity in hepatic and cardiac tissues, as evidenced by changes in LPO was studied to reveal the drug-induced tissue damage, if existing.

2. Investigation and results

In the present study the effect of an ethanolic extract of *T. arjuna* bark was investigated in L-T_4 induced hyperthyroid and euthyroid rats. Estimation of serum thyroxine

Table 1: Effects of *T. arjuna* (TA) bark extract (42.84, HD and 21.42 mg/kg, LD) for two weeks on total serum T_3, T_4 concentrations (ng/ml), hepatic and cardiac LPO (nM MDA formed/h/mg protein) and heart/body weight (mg/g) in T_4 induced hyperthyroid and in normal euthyroid (Ctrl) female rats

Groups	T_3	T_4	Cardiac LPO	Hepatic LPO	Heart wt./b. wt.
Ctrl	0.81 ± 0.05	112.57 ± 4.13	1.54 ± 0.15	0.96 ± 0.10	3.64 ± 0.15
T_4 + DW	$2.05^{z} \pm 0.15$	$205.42^{z} \pm 6.32$	$3.69^{z} \pm 0.37$	$1.81^{x} \pm 0.29$	4.30 ± 0.19
T_4 + PTU	$0.934^{c} \pm 0.058$	$113.14^{c} \pm 5.41$	$2.34^{a} \pm 0.266$	$1.10^{a} \pm 0.098$	3.90 ± 0.04
T_4 + TA(HD)	$1.06^{c} \pm 0.062$	$159.4^{c} \pm 6.21$	$0.903^{c} \pm 0.076$	$0.880^{b} \pm 0.104$	4.25 ± 0.22
T_4 + TA(LD)	$1.52^{b} \pm 0.089$	$182.85^{a} \pm 7.24$	$1.47^{c} \pm 0.274$	$1.08^{a} \pm 0.084$	4.51 ± 0.15
PTU	$0.48^{z} \pm 0.03$	$64.14^{z} \pm 5.87$	1.02 ± 0.25	$0.456^{z} \pm 0.054$	3.42 ± 0.09
TA(HD)	$0.55^{x} \pm 0.08$	$77.57^{y} \pm 7.37$	1.27 ± 0.196	$1.40^{y} \pm 0.086$	3.74 ± 0.11
TA(LD)	0.61 ± 0.85	$82.28^{y} \pm 8.47$	$0.40^{z} \pm 0.04$	$1.35^{x} \pm 0.12$	3.68 ± 0.23

Data are means $\pm$ SEM. ($n = 7$); a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$ as compared to the respective values of T_4 treated group. x, $p < 0.05$; y, $p < 0.01$; z, $p < 0.001$ as compared to the respective control values

Table 2: Effects of a suboptimal dose (10.11 mg/kg) of *T. arjuna* (TA) bark extract for two weeks on total serum T_3, T_4 concentrations (ng/ml), hepatic and cardiac LPO (nM MDA formed/h/mg protein) and heart/body weight (mg/g) in female rats

Groups	T_3	T_4	Cardiac LPO	Hepatic LPO	Heart wt./b.wt.
Ctrl	0.70 ± 0.01	78.28 ± 9.04	1.46 ± 0.25	1.08 ± 0.29	3.13 ± 0.06
TA	0.62 ± 0.06	71.42 ± 15.01	1.24 ± 0.25	1.73 ± 0.15	3.31 ± 0.13

Data are means ±SEM (n = 7)

(T_4), triiodothyronine (T_3) concentrations, hepatic and cardiac lipid peroxidation (LPO) and heart and body wt. ratio were considered as the main parameters.

Following L-T_4 (0.5 mg/kg) administration, a significant increase in all parameters including serum T_3 and T_4 concentrations, hepatic and cardiac LPO were found, while in heart and body weight ratio, a marginal increase was observed. However, simultaneous administration of 21.42 (LD) and 42.84 mg/kg (HD) of the plant extract (Bharani et al. 2002) in L-T_4 treated animals significantly decreased the concentration of both the thyroid hormones and tissue LPO (Table 1).

In euthyroid animals also the higher dose of plant extract decreased the concentration of serum T_4 and T_3. However, it increased hepatic LPO as compared to the respective control value. Following the administration of a lower dose of the plant extract there was a significant decrease in T_4 concentration and in cardiac LPO, but an increase in hepatic LPO was observed.

PTU administration to hyperthyroid animals decreased the concentration of both the thyroid hormones and tissue LPO. The equivalent dose of PTU in euthyroid animals also decreased both the thyroid hormones and hepatic LPO as compared to the respective control values. When a suboptimal dose of the plant extract was tried, no significant effect was observed in any of the parameters studied (Table 2).

3. Discussion

While exogenous L-T_4 enhanced the serum thyroid hormone concentration, heart/body wt. ratio as well as cardiac LPO as in accordance with the earlier observations made by some other workers (Civelek et al. 2001; Hu et al. 2003), simultaneous administration of 42.84 and 21.42 mg/kg of *Arjuna* bark extract decreased thyroid hormone concentrations in L-T_4 induced hyperthyroid animals indicating antithyroidal potential of the plant extract. A parallel decrease in cardiac lipid peroxidation observed in these hyperthyroid animals receiving the plant extract also suggests its cardio-protective role as reported earlier (Karthikeyan et al. 2003; Ali et al. 2003).

Although some plant extracts have been reported to ameliorate cardiovascular problems (Van and Dall 1983), so far in only one of these plants, thyroid function has been studied (Panda and Kar 2000a). In fact, regarding the role of *Arjuna* bark extract in regulating thyroid function, the present one appears to be the first report.

As there was a decrease in thyroid function as well as in cardiac LPO by the administration of the test plant extract, it is possible that the cardio-protective role of this plant extract might have been mediated through an inhibition in thyroid function. This possibility is further supported by the fact that hyperthyroidism very often causes tachycardia, hypertension, and cardiac hypertrophy (Donatelli et al. 2003; Watanabe et al. 2003; Wald et al. 2003; De-Tommasi et al. 2003; Hu et al. 2003).

The present plant extract was also found to inhibit thyroid hormone concentration in euthyroid animals at both the doses, suggesting that the *Arjuna* bark extract may cause hypothyroidism in normal/euthyroid subjects. When hepatic LPO was studied, although a decrease was observed in hyperthyroid animals following the administration of the plant extract, in euthyroid animals, it was increased, indicating its oxidative (harmful) nature in normal/healthy individuals. Somewhat similar observations have been made earlier in relation to the hepatic lipid peroxidation in euthyroid animals, where also peroxidative effects of some plant extracts were observed when administered in higher concentrations (Panda and Kar 1998, 2000a, b).

The observed antithyroidal role of the *Arjuna* extract in hyperthyroid animals is similar to that of another plant extract (Panda and Kar 2000a). Since both the thyroid hormones, T_3 and T_4 were decreased by the test material, it appears that the *Arjuna* extract is capable of inhibiting thyroid function both at glandular (major source of T_4 synthesis) as well as at the peripheral level of T_4 to T_3 conversion, the principal pathway of T_3 generation.

It was interesting to note both the doses of the test plant material increased hepatic LPO, in euthyroid animals, suggesting its toxic nature. Although a sub optimal dose (10.11 mg/kg) of the extract did not cause any liver toxicity, it does not appear to be useful, as no significant effect was noticed in any of the parameters studied.

In conclusion our findings clearly indicate that moderate doses of *Arjuna* bark extract may potentially ameliorate L-T_4 induced cardiac lipid peroxidation possibly through inhibiting thyroid function in female rats. However, a higher concentration may be hepatotoxic and therefore, intake of high amounts of *Arjuna* bark extract should be avoided. It is further suggested that this plant extract should not be consumed by normal/healthy individuals, as it may cause hepatotoxicity as well as hypothyroidism.

4. Experimental

4.1. Animals

Wistar adult female rats, weighing 150 ± 5 g, housed in polypropylene cages, were maintained at constant temperature (27 ± 1 °C) and photo schedule (14 h light and 10 h dark) with the provision of laboratory feed (Gold Mohur feed, Hindustan Lever Ltd., Mumbai, India) and water a*d libitum*.

4.2. Chemicals

Thiobarbituric acid (TBA), pyrogallol, hydrogen peroxide, diethylene-triamine penta acetic acid, L-T_4, PTU and sodium dodecyl sulphate (SDS) were obtained from E. Merck (India) Ltd., Bombay, India. The Bhabha Atomic Research Center (BARC), Mumbai, India, supplied radioimmunoassay (RIA) kits for the estimation of total T_3 and T_4. All other chemicals were of reagent grade and obtained from Loba chemie, Mumbai, India.

4.3. Preparation of plant extract

The dried *Arjuna* bark was crushed to powder and then extracted with 90% (v/v) ethyl alcohol at room temperature following the earlier method (Bharani et al. 2002). Drying was carried out at 60 °C under vacuum. The dried material was powdered and solubilized it in distilled water for administration.

4.4. Experimental design

The experiments were conducted after an acclimation period of one week.

4.4.1. Experiment 1

Fifty-six healthy rats were divided into eight groups of seven each and the body weight of each animal was recorded. Group one animals receiving the vehicle (distilled water, 0.1 ml/d/animal) served as control. While group two, three, four and five received 0.5 mg/kg, s.c. of L-T_4 for inducing hyperthyroidism (Panda and Kar 2000a), group six, seven, eight received only PTU (10 mg/kg, i.p.), a higher dose of the plant extract (42.84 mg/kg, p.o.) and a lower dose of the plant extract (21.42 mg/kg, p.o.), respectively. In addition to an equivalent amount of T_4, group two, three, four, and five received distilled water, PTU, higher dose of plant extract and lower dose of plant extracts respectively. Treatments were continued for two weeks. On the day of termination the final body weight of each animal was recorded, blood was collected from the overnight fasted animals and the serum samples were stored at $-20\,^{\circ}C$ for radioimmunoassay (described later) for the estimation of total T_3 and T_4 concentrations, as performed routinely in our laboratory (Panda and Kar 2000a). After exsanguinations, the liver and the heart were removed quickly, freed from blood clots, weighed and processed for the LPO study (Ohkawa et al. 1979).

4.4.2. Experiment 2

As the test drug exhibited hepatotoxicity in euthyroid animals even at the lower dose (21.42 mg/kg), a second trial was made with a sub-optimal dose (10.11 mg/kg) considering the same parameters as studied in experiment 1 to reveal the toxicity of this dose.

4.5. Biochemical assay of hepatic LPO

For the evaluation of LPO activity, the liver was homogenized in 10% (w/v) ice-cold phosphate buffer (0.1 M, pH 7.4) and the homogenate was centrifuged at $15{,}000 \times g$ for 30 min. LPO was studied using our standard protocol (Ohkawa et al. 1979). In brief, it was determined by the reaction of TBA in which malondialdehyde (MDA), a product formed due to the peroxidation of lipids was estimated. The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient, $E = 1.56 \times 10^5$), using a Shimadzu UV-1601 spectrophotometer. LPO was finally expressed as nM MDA formed/h/mg protein.

4.6. Radio-immunoassay (RIA) of thyroid hormones

Total circulating T_3 and T_4 were estimated by radioimmunoassay in serum samples following the protocol provided in the RIA kits supplied by BARC, Mumbai, India as routinely followed in our laboratory (Panda and Kar 1998, 2000a, b). In brief, RIA was performed using Tris hydroxymethyl amino methane (THAM) buffer (0.14 M containing 0.1% gelatin; pH 8.6). The antisera, specific hormone standards, radio labeled hormones (I^{125} T_4 and I^{125} T_3) and the control sera were reconstituted with assay buffer/double distilled water. The reaction mixture comprised of standard/sample, buffer, radio labeled hormone and the respective antibody. The reaction mixture was incubated at 37 °C (for 30 min for T_4 and 45 min for T_3). The incubation was terminated by the addition of PEG. The tubes were then centrifuged at $2000 \times g$ for 20 min. After decanting the supernatant, the traces of liquid were removed with the help of filter paper wicks without disturbing the precipitate. Finally the tubes were subjected to radioactivity counting for 1 min (CPM) using an I^{125} gamma counter. A set of quality control sera was also run with each assay.

4.7. Statistical analysis

Data are expressed as mean ±S.E.M. For statistical evaluation of the data, analysis of variance (ANOVA) and the student's t-test were used.

Acknowledgements: Financial support from Council of Scientific and Industrial Research (CSIR) for Research Associate and Junior Research Fellowship grants to S. Panda and R. Jatwa respectively, is gratefully acknowledged.

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