Chemico-Biological Interactions 177 (2009) 254–258

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Protective role of *Mangifera indica*, *Cucumis melo* and *Citrullus vulgaris* peel extracts in chemically induced hypothyroidism

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ARTICLE INFO

Article history:
Received 20 May 2008
Received in revised form 8 November 2008
Accepted 10 November 2008
Available online 18 November 2008

Keywords:
Citrullus vulgaris
Cucumis melo
Hypothyroidism
Lipid peroxidation
Mangifera indica
Thyroid hormones

ABSTRACT

An investigation was made to evaluate the pharmacological importance of fruit peel extracts of *Mangifera indica* (MI), *Citrullus vulgaris* (CV) and *Cucumis melo* (CM) with respect to the possible regulation of tissue lipid peroxidation (LPO), thyroid dysfunctions, lipid and glucose metabolism. Pre-standardized doses (200 mg/kg of MI and 100 mg/kg both of CV and CM), based on the maximum inhibition in hepatic LPO, were administered to Wistar albino male rats for 10 consecutive days and the changes in tissue (heart, liver and kidney) LPO and in the concentrations of serum triiodothyronine (T_3), thyroxin (T_4), insulin, glucose, α-amylase and different lipids were examined. Administration of three test peel extracts significantly increased both the thyroid hormones (T_3 and T_4) with a concomitant decrease in tissue LPO, suggesting their thyroid stimulatory and antiperoxidative role. This thyroid stimulatory nature was also exhibited in propylthiouracil (PTU) induced hypothyroid animals. However, only minor influence was observed in serum lipid profile in which CM reduced the concentrations of total cholesterol and low-density lipoprotein-cholesterol (LDL-C), while CV decreased triglycerides and very low-density lipoprotein-cholesterol (VLDL-C). When the combined effects of either two (MI + CV) or three (MI + CV + CM) peel extracts were evaluated in euthyroid animals, serum T_3 concentration was increased in response to MI + CV and MI + CV + CM treatments, while T_4 level was elevated by the combinations of first two peels only. Interestingly, both the categories of combinations increased T_4 levels, but not T_3 in PTU treated hypothyroid animals. Moreover, a parallel increase in hepatic and renal LPO was observed in these animals, suggesting their unsafe nature in combination. In conclusion the three test peel extracts appear to be stimulatory to thyroid functions and inhibitory to tissue LPO but only when treated individually.

1. Introduction

Fruit peels are rich in polyphenolic compounds, flavonoids, ascorbic acid and number of other biologically active components having positive influence on health [1–4]. Some fruit peels are also reported for various biological activities in normal healthy animals and experimental models [2,3,5–10]. However, scientific investigations on fruit peels in relation to regulation of thyroid function are negligible.

On melon fruits, some reports are available on its antioxidative, anti-inflammatory, platelet inhibitory, urease inhibitory, diuretic and anthelmintic potential [11–16]. Rind of watermelon is also known as a potential source of antioxidative, vasodilatory and immunomodulatory activities [17–19]. Similarly, officinal parts of the mango, such as stem bark, leaves and fruit are known for various biomedical applications including antioxidative or free radical scavenging [20,21]; anti-inflammatory [22,23]; anti-allergic [24]; cardio-protective [25]; anticancer [26]; hepatoprotective [27,28]; analgesic and immunomodulator [29,30] activities.

Despite these reports, primarily on the whole fruits, there is a paucity of scientific literature on the importance of their peels. In recent past, we also reported the pharmacological effects of some other fruit peel extracts against cardiovascular problems, thyroid abnormalities and diabetes mellitus [10,31–33]. Therefore, for the first time an attempt has been made to evaluate the peel extracts of Mango (*Mangifera indica*, MI), Watermelon (*Citrullus vulgaris*, CV) and Melon (*Cucumis melo*, CM) with respect to their efficacy in altering the serum levels of thyroid hormones, glucose, insulin, α-amylase, different lipids and tissue lipid peroxidation (LPO) in normal healthy and PTU (propylthiouracil) induced hypothyroid rats, which may be characterized by the decreased serum levels of both the thyroid hormones (T_3 and T_4) with a concomitant increase in thyroid stimulating hormone (TSH) [34]. It is also known to reduce the oxidative damage in various tissues, due to hypometabolism, decreased production of reactive oxygen metabolites and enhancement of antioxidant mechanisms [35].

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doi:10.1016/j.cbi.2008.11.006

2. Materials and methods

2.1. Chemicals

Sodium dodecyl sulphate, 2-thiobarbituric acid and propylthiouracil were purchased from E. Merk Ltd., India, while, radioimmunoassay (RIA) kits for the estimation of different hormones were supplied by Bhabha Atomic Research Center, Mumbai, India.

2.2. Animals

Healthy colony bred Wistar albino male rats (140 ± 10 g) were considered for the study. These were maintained in polypropylene cages ($43\,cm \times 27\,cm \times 25\,cm$ with floor area of $165.85\,cm^2$/animal). Seven animals were placed in each cage and were maintained under constant temperature ($23 \pm 2\,^{\circ}C$) and photo schedule (14 h light: 10 h dark). They were provided with commercial rodent feed comprised of protein, 20–22%; oil, 3.5%; crude fiber, 4%; ash, 6%; calcium, 1%; phosphorous, 0.5%; lysine, 1.2% and methionine, 0.9% with metabolic energy 3000 kcal (Gold Mohur feeds Ltd., New Delhi, India) *ad libitum* and had free access to drinking water. Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India and Departmental Ethical Committee for handling and maintenance for experimental animals were followed.

2.3. Plant material

Fresh fruits of Mango (*Mangifera indica*, Family-Anacardiaceae), Melon (*Cucumis melo*, Family-Cucurbitaceae) and Watermelon (*Citrullus vulgaris*, Family-Cucurbitaceae) were purchased from the local market. They were peeled off mechanically and good quality peels were air dried under shadow and then grounded into fine powder by pulverization. For the preparation of methanolic extract of mango (MI), melon (CM) and watermelon (CV) peels; procedure described by earlier workers was used [7]. In brief, 100 g peel powder was extracted with 600 ml of methyl alcohol at 30 °C for 4 h with continuous stirring in a magnetic stirrer and then filtered. The filtrate was dried and stored for future use.

2.4. Experimental design

2.4.1. Experiment 1: study of peel extracts on normal healthy animals

A preliminary study was carried out to standardize the doses of peel extracts, where hepatic LPO was considered as an end parameter. From this study it was observed that out of four doses (50, 100, 200 and 300 mg/kg), maximum inhibition in hepatic LPO was observed at 200 mg/kg of MI, and 100 mg/kg of CV and CM. Twenty-eight healthy rats were divided into four groups of seven each. They were acclimatized in animal house for a week before experimentation. Animals of group II, III and IV were administered (p.o.) with MI (200 mg/kg), CM (100 mg/kg) and CV (100 mg/kg) extracts, respectively for 10 consecutive days. However, animals of group I (control) were administered with equivalent amount of distilled water in which the drug was finally prepared for administration. Peel extracts or the vehicle was administered by gastric intubations at a fixed time (1000–1100 h) of the day to avoid circadian variations, if any.

2.4.2. Experiment 2: effects of peel extracts on PTU induced hypothyroid animals

Thirty-five healthy rats were divided into five groups of seven each. Animals of group II were treated with 10 mg/kg/day PTU (i.p.), while animals of group III, IV and V were also administered with PTU and 200/100/100 mg/kg p.o. peel extracts of MI/CV/CM, respectively. Animals of group I receiving equivalent amount of distilled water in which the drug or PTU was finally prepared, served as control group. Rests of the experimental conditions were similar to the previous one. Experiment was terminated after 10 days of treatment.

2.4.3. Experiment 3: effects of peel extracts in combination (MI + CV + CM or MI + CV) on euthyroid and PTU induced hypothyroid animals

Combinations of MI + CV + CM and MI + CV were selected on the basis of their higher thyroid stimulatory potential. Forty-two healthy rats were divided into six groups of seven each. Animals of group II were treated with 10 mg/kg/day of PTU (i.p.) while, animals of group III and IV were administered with equivalent amount of PTU along with the 200, 100 and 100 mg/kg, p.o. peel extracts of MI, CV and CM in group III or with 200 and 100 mg/kg, p.o. of MI and CV peel extracts in group IV. Animals of group V and VI received equivalent amount of MI + CV + CM and MI + CV, respectively. Group I receiving equivalent amount of distilled water served as a control. Experiment was continued for 10 days before termination.

2.5. Serum and tissue preparation

On the last day (11th day) of experimentation, overnight fasted animals were killed by cervical dislocation after exposing them to mild ethyl ether anesthesia. Blood samples were collected, allowed to clot and centrifuged to get a clear serum, which was stored at $-20\,^{\circ}C$ until used for different biochemical estimations. Liver, heart and kidney were removed quickly, cleaned and washed twice with phosphate buffered saline (0.1 M, pH 7.4), homogenized and immediately processed for the estimation of LPO.

2.6. Biochemical estimations in serum

Fasting serum glucose concentration was measured by the glucose oxidase/peroxidase method of Trinder [36], where 4-aminoantipyrine and phenol reacted with glucose to yield a red colour complex, as followed earlier in our [37,38]. While for the estimation of serum total cholesterol and HDL-C, method of Allain et al. [39] was followed. Triglyceride and the activity of α-amylase activity were studied by using the protocols of Fossati and Lorenzo [40] and Ranson [41], respectively. LDL-C and VLDL-C were calculated by the formula of Friedwald et al. [42].

Total circulating T_3 and T_4 were estimated by radioimmunoassay (RIA) in serum samples following the protocol provided in the RIA kits supplied by Bhabha Atomic Research Centre (BARC), Mumbai, India, as done earlier in our laboratory [37,38,43]. Assay of serum insulin was done following the earlier protocol of our laboratory [10]. A set of quality control sera of rats was also run with the assay.

2.7. Estimation of tissue lipid peroxidation (LPO)

For the evaluation of LPO, heart, liver and kidney tissues were homogenized in 10% (w/v) ice-cold phosphate buffered saline (0.1 M, pH 7.4), centrifuged at $2000 \times g$ for 30 min and the supernatant was used for the assay [10,37,43]. In brief, LPO was determined by the reaction of 2-thiobarbuturic acid with thiobarbituric acid reactive substances (TBARS) including malondialdehyde (MDA), a major product formed due to the peroxidation of lipids. Amount of TBARS was measured by taking the absorbance at 532 nm (extinction coefficient, $E = 1.56 \times 10^5$), using a Shimadzu UV-160 spectrophotometer. LPO was finally