

Lagenaria siceraria Peel Extract in the Regulation of Hyperthyroidism, Hyperglycemia and Lipid Peroxidation in Mice

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ABSTRACT

The potential of *Lagenaria siceraria* peel extract in the regulation of lipid peroxidation, hyperthyroidism and hyperglycemia has been evaluated in mice. In an *in-vitro* study the quenching potential of the peel extract (5-100 µg/ml) on the 1,1-diphenyl-2-picrylhydrazyl (DPPH)-dependent free radicals was examined. Antioxidative potential was also studied in carbon tetrachloride (CCl₄)- and hydrogen peroxide (H₂O₂)-induced lipid peroxidation (LPO) in liver tissues. In another experiment, an *in-vivo* study was performed considering three different concentrations of the test peel extract to select its most effective and safe dose for the regulation of hepatic LPO, thyroid function and glucose metabolism. Out of 50, 100 and 200 mg/kg of the peel extract, 100 mg/kg was found to be the most effective and safe concentration, as it could inhibit the levels of serum thyroxine (T₄), triiodothyronine (T₃) and glucose as well as hepatic LPO. Considering this dose, finally the antithyroidal, antiperoxidative and glucose inhibitory potential of the peel extract were tested in T₄-induced hyperthyroid animals. After 21 days of treatment, a decrease in the concentrations of serum thyroid hormones, glucose as well as in hepatic LPO with a parallel increase in antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) indicated the efficacy of the test peel in the amelioration of hyperthyroidism, hyperglycemia and hepatic lipid peroxidation.

Keywords: antiperoxidation, bottle gourd, free radicals, thyroxine, triiodothyronine

Abbreviations: CAT, catalase; GSH, glutathione; LPO, lipid peroxidation; SOD, superoxide dismutase; T₃, triiodothyronine; T₄, thyroxine

INTRODUCTION

The thyroid is considered as one of the most important endocrine organs. As hormones of this gland regulate almost all body functions, their abnormal production very often results in complex health disorders including diabetes, hypertension and cardiovascular problems. Although some synthetic thyroid regulating compounds such as Eltroxin[®] and Neomarkazole[®] are available, their prolonged use may have some side effects such as skin rash, hair loss, stomach upset, vomiting, abnormal sensations, muscle pain, drowsiness, dizziness and decreased white blood cells (Satoskar *et al.* 1999). There is a need for safe, alternative medicine for the regulation of thyroid problems. Botanicals are very often considered as safe and economic therapeutics. Some herbal extracts including that of *Withania somnifera*, *Bauhinia purpuria*, *Commiphora mukul*, *Rauwolfia serpentina*, *Convolvulus pluricaulis* and *Annona squamosa* have also been investigated with respect to the regulation of hypo- and hyperthyroidism (Kar and Panda 2004, 2005). Some of the recent reports from our laboratory also indicate the beneficial effects of peels from some fruits such as *Citrus sinensis*, *Musa paradisiaca* and *Punica granatum* (Parmar and Kar 2007). However, on the efficacy of the vegetable peels, information is negligible (Nara *et al.* 2006), despite the fact that few vegetables are goitrogenic in nature (Chandra *et al.* 2004).

Lagenaria siceraria (family, *Cucurbitaceae*), commonly known as bottle gourd, is an annual herb that is consumed as a vegetable, sometimes along with its peel in most Asian countries. The whole fruit is reported to be useful in Ayurveda, the Indian system of medicine for the treatment of insomnia and hyperlipidemia (Nadkarni and Nadkarni 1976;

Ghule *et al.* 2006a). It has also been attributed with anti-hepatotoxic, analgesic and anti-inflammatory activities (Shirwaikar and Sreenivasan 1996; Ghule *et al.* 2006b). Moreover, the phytochemical analysis of this vegetable has indicated the presence of sterols, flavonoids, cucurbitacin, saponins, and polyphenolics that are believed to have antioxidative potential (Ghule *et al.* 2006a). However, with reference to the importance of its peel, nothing has been investigated so far. Therefore, in the present investigation an attempt was made to explore the possible antiperoxidative role of this peel extract considering lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as the main parameters. As thyroid abnormalities are also related with oxidative damage and hepatotoxicity (Panda and Kar 2001), we further hoped to reveal its potential, if any, in the regulation of thyroid problems.

MATERIALS AND METHODS

Plant material

Fresh bottle gourds were collected from the local market, Indore (M.P., India). Peels were then removed, shade-dried for one week and pulverized with the help of an electric grinder to get a free flowing powder. The dry powder was subjected to extraction with 50% ethanol (v/v) at room temperature for 24 h (Ghule *et al.* 2006a). The extract was filtered through Whatman filter No. 1 paper and was dried at 37°C to get a dry powder (yield = 13% (w/w) of the starting raw material). The powder was dissolved in double-distilled water to prepare a dose equivalent to 200 mg/kg body weight for use in our experiment. This dose was based on a previous study (Ghule *et al.* 2006a).

Chemicals

Tris and thiobarbituric acid (TBA) were obtained from E. Merck, Mumbai, India. Pyrogallol, sodium dodecyl sulphate (SDS) and all other chemicals were purchased from Loba Chemicals, Mumbai, India. Radioimmunoassay (RIA) kits for T₃ and T₄ were supplied by Bhabha Atomic Research Centre (BARC), Mumbai, India.

Experimental design

Three different experiments were performed.

Experiment 1: Antioxidative effect – an *in vitro* study

Scavenging of DPPH free radicals

To assess the direct free radical scavenging activity, if any, stable 1, 1-diphenyl 2-picryl hydrazyl radical (DPPH) was used (Koleva *et al.* 2002). In brief, different concentrations (5-100 µg/ml) of the peel extract were added at an equal volume of methanolic solution of DPPH (100 µM). After 15 min incubation at room temperature, the absorbance was read at 517 nm. Quercetin was used as the standard control and the IC₅₀ value was calculated as the concentration of sample which could scavenge 50% of DPPH radicals.

Inhibition of CCl₄- and H₂O₂-induced hepatic lipid peroxidation

This *in-vitro* study was performed using liver slices of colony-bred Wistar albino male rats obtained from our departmental animal house. Five different concentrations of peel extract were prepared which were then used to study their efficacy in the inhibition of carbon tetrachloride (CCl₄)- or hydrogen peroxide (H₂O₂)-induced LPO according to Stocks and Dormandy (1971) and Shinya *et al.* (2007). In brief, two male rats were sacrificed by decapitation, the liver from each was removed, washed three times with 0.1 M phosphate buffered saline (PBS, 1:9 (v/v), pH 7.4) and then dried with filter paper. The separated liver was cut into 0.5 mm³ slices and placed into separate test tubes containing 5 ml of Krebs-Ringer buffer pH. 7.4 (Wormser *et al.* 1990). The liver slices were incubated with different concentrations of peel extracts (5-100 µg/ml) along with CCl₄ (dissolved in DMSO, 1:4 (v/v)) or H₂O₂ (100 µl of 10 µM H₂O₂) for 2 hrs at 37°C in a thermostatically controlled water bath. After incubation, tubes were subjected to centrifugation at 1000 × g for 5 min and the supernatant was discarded. The liver slices were then homogenized in PBS buffer (pH 7.4) to yield a 10% homogenate, which was finally subjected to the lipid peroxidation study (described below).

Experiment 2. Effects on thyroid hormones, glucose and lipid peroxidation – An *in vivo* study

As in the previous experiment (in *in-vitro* study), *L. siceraria* peel was found to exhibit free radical scavenging /LPO inhibiting property, its antioxidant potential was also evaluated in *in-vivo* condition in mice. Simultaneously, changes in the serum concentrations of thyroid hormones and glucose were examined in order to reveal its possible regulatory property in thyroid functions and glucose metabolism. For this, healthy Swiss albino mice of about 2 months old (28 ± 2 g) were obtained from the departmental animal house and were acclimatized in temperature (27 ± 1°C) and light controlled (14 hr light: 10 hr dark) room with the provision of food (Gold Mohur animal feed, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. Mice were divided into four groups of seven each and initial body weight of each one was recorded. While group 1 received 0.1 ml of double distilled water (orally) and served as control, animals of group 2, 3 and 4 were administered with 0.1 ml of the peel extract equivalent to the dose of 50, 100 and 200 mg/kg respectively. The drug administration was done orally with the help of a feeding needle every day between 10 and 11 h to avoid circadian interference. The treatment was continued for 21 days. At the end, alterations in the concentrations of serum thyroid hormones, glucose and in hepatic LPO were studied as described later.

Experiment 3. Effects on hyperthyroid animals

As the results of experiment 2 indicated the thyroid hormone and glucose lowering potential of the test peel extract, a trial was made in L-T₄-induced hyperthyroid animals in order to ascertain the anti-thyroidal or anti-hyperglycemic effects of the peel extract.

Under this experiment, 21 mice were divided into three groups of seven each and initial body weight of each one was recorded. While group 1 animals receiving 0.1 ml of double distilled water (orally) served as control, those of group 2 and 3 were rendered hyperthyroid by daily administration of L-T₄ (s.c.) at a pre-standardized dose of 500 µg/ kg/day for 12 days (Panda and Kar 2001). After the 12th day, group 2 animals received 0.1 ml of distilled water, while group 3 was treated with 0.1 ml of the test peel extract at a dose of 100 mg/kg orally with the help of a feeding needle. Drug or the vehicle was administered between the 10th and 11th hour of the day to avoid circadian interference and the treatment was continued for 21 days.

Biochemical estimations

At the end of the experiment, overnight fasted mice were weighed and were sacrificed by cervical dislocation. Blood from each one was collected, allowed to clot and then centrifuged to get the clear serum. Serum samples were stored at -20°C for the estimation of blood glucose and thyroid hormones. Liver was quickly removed, cleaned and then homogenized in phosphate buffer (0.1 M, pH 7.4) with the help of a motor-driven teflon homogenizer. The homogenate was centrifuged at 15,000 × g at 4°C for 30 min to obtain a clear supernatant, which was used for the estimation of LPO, SOD, CAT, GSH and G-6-Pase activities that are routinely done in our laboratory (Panda and Kar 2001; Parmar and Kar 2007). In brief, LPO 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 X 10⁵), using a Shimadzu UV-160 spectrophotometer. LPO was finally expressed as nM MDA formed/h/mg protein (Ohkawa *et al.* 1979). The activity of hepatic SOD was estimated by measuring the percentage inhibition of the pyrogallol autooxidation by the enzyme (Marklund and Marklund 1974). One unit of SOD is defined as the enzyme activity that inhibits the autooxidation of pyrogallol by 50%. Catalase activity was estimated by the method of Aebi (1983) and was expressed as µM of H₂O₂ decomposed/min/mg protein. For GSH content the method described by Ellman (1959) was followed and finally expressed as µg GSH/mg protein. Assays of hepatic G-6-pase and protein were measured by our routinely used methods (Lowry *et al.* 1951; Baginski 1974). The concentration of serum glucose was estimated by the enzymatic method using kits from Qualigens Fine Chemicals, Glaxo India Ltd, Mumbai, India.

Radioimmunoassay of thyroid hormones

Total circulating T₃ and T₄ were estimated by radioimmunoassay (RIA) in serum samples following the protocol provided in the RIA kits, as routinely done in our laboratory (Panda and Kar 2003; Parmar and Kar 2007). In brief, RIA was performed using tris-hydroxymethyl aminomethane buffer (0.14 M, containing 0.1% gelatin; pH 8.6). The antisera-specific hormone standards, radiolabelled hormones (I¹²⁵T₄ and I¹²⁵T₃) and the control sera were reconstituted with assay buffer/double distilled water. The reaction mixture comprised of standard /sample, buffer, radiolabelled hormone and the respective antibody, which was incubated at 37°C (30 min for T₄ and 45 min for T₃). Incubation was terminated by the addition of polyethylene glycol. Tubes were then centrifuged at 2000 × g for 20 min. After decanting the supernatant 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 using the I¹²⁵ gamma counter. A set of quality control sera was also run with each assay.

Animal ethics

Standard ethical guidelines of the Committee for the Purpose of Control and Supervision on Experiments in Animals, Ministry of Environment and forest, Government of India, were followed. Before starting the investigation, the approval of the experimental design by the departmental (School of Life Sciences, DA University, Indore, India) Ethical Committee for Handling and Maintenance for Experimental was obtained.

Statistics

Data are expressed as means \pm S.E.M. and were analyzed by the analysis of variance (ANOVA) followed by *Post hoc* Newman-Keul's Multiple Comparison Test by using trial version of Prism software for windows.

RESULTS

Antioxidative effect: An *in-vitro* study

Results obtained by the DPPH discoloration test (**Table 1**) indicated the free radical scavenging activity of the peel extract in a concentration-dependent manner up to 100 $\mu\text{g/ml}$. The standard reference quercetin also exhibited a free radical scavenging activity at the concentrations from 5 to 100 $\mu\text{g/ml}$. However, the maximum DPPH radical inhibition was 85% and 95% for *Lagenaria* extract and quercetin, respectively. The IC_{50} value was 44.61 $\mu\text{g/ml}$ for the test peel that was comparable to the standard, quercetin which had an IC_{50} value of 50.62 $\mu\text{g/ml}$.

CCl_4 -induced lipid peroxidation was also inhibited in a dose-dependent manner by the test peel extract at all concentrations used (5-100 $\mu\text{g/ml}$, **Table 2**) which was comparable to the reference compound, BHA (Leontowicz *et al.* 2003). However, the highest inhibition was observed at 100 $\mu\text{g/ml}$ of both BHA as well as of the test peel extract. Somewhat similar results were obtained with respect to H_2O_2 -induced lipid peroxidation, where the highest inhibition was observed in 50 $\mu\text{g/ml}$ of BHA and 100 $\mu\text{g/ml}$ of the test peel extract. IC_{50} values for the test peel and BHA were 10 and 5 $\mu\text{g/ml}$, respectively. A similar inhibition was also observed in H_2O_2 -induced hepatic LPO by the peel extract in a dose-dependent manner and the IC_{50} value was 12 and 22 $\mu\text{g/ml}$ for the test peel and BHA, respectively.

Effects on thyroid hormones, glucose and lipid peroxidation: An *in-vivo* study

Serum T_3 , T_4 and glucose concentrations were significantly

Table 1 DPPH free radical scavenging activity (% inhibition) by different concentrations of *Lagenaria siceraria* peel extract: a comparison with the standard, quercetin. IC_{50} values: 50.62 and 44.61 $\mu\text{g/ml}$ for quercetin and the test peel extract, respectively. (n = 3)

Concentrations of peel extract ($\mu\text{g/ml}$)	Quercetin	Peel extract
5	16.72 \pm 2.22	11.74 \pm 0.41
10	25.70 \pm 1.37	19.96 \pm 0.75 c
25	46.41 \pm 2.50	36.67 \pm 0.68 b
50	54.60 \pm 0.87	70.62 \pm 1.26 a
100	75.87 \pm 1.25	85.19 \pm 0.42 b

Values are means \pm SEM.

a: $p < 0.001$, b: $p < 0.01$ and c: $p < 0.05$ as compared with the respective control values.

Table 2 *In vitro* free radical scavenging activity (% inhibition in LPO as compared to BHA) of the test peel extract (PE) in CCl_4 - and H_2O_2 -treated rat liver slices. (n = 3)

Dose ($\mu\text{g/ml}$)	Treatment			
	CCl_4 + BHA	CCl_4 + PE	H_2O_2 + BHA	H_2O_2 + PE
5	49.97 \pm 1.57	37.16 \pm 1.33 b	39.71 \pm 1.74	25.95 \pm 4.68
10	52.17 \pm 1.13	44.02 \pm 2.17 c	42.21 \pm 1.51	48.47 \pm 3.21
25	63.36 \pm 2.17	60.84 \pm 2.77	56.30 \pm 2.90	57.70 \pm 4.00
50	79.17 \pm 3.15	70.14 \pm 3.80	73.34 \pm 2.50	67.12 \pm 3.11
100	89.35 \pm 2.76	85.26 \pm 3.01	80.35 \pm 2.76	72.22 \pm 4.70

Values are means \pm SEM.

b: $p < 0.01$ and c: $p < 0.05$ as compared with the respective control values.

CCl_4 + BHA: carbon tetrachloride-treated and butylated hydroxy anisole.

CCl_4 + PE: carbon tetrachloride-treated and peel extract.

H_2O_2 + BHA: hydrogen peroxide-treated and butylated hydroxy anisole.

H_2O_2 + PE: hydrogen peroxide-treated and peel extract.

decreased by the administration of 100 mg/kg of the test peel extract (**Table 3**). Hepatic LPO was also significantly reduced in these animals. However, other two doses, 50 and 200 mg/kg could not bring about any significant effect.

Effects in hyperthyroid animals

While a significant increase in serum T_3 and T_4 concentrations and in G-6-pase activity was observed in T_4 -induced hyperthyroid mice, following the administration of 100 mg/kg of the peel extract together with T_4 , there was a significant decrease in the values of all three parameters. L- T_4 administration also significantly increased hepatic (**Table 4**). LPO and serum glucose concentration and was accompanied by a parallel decrease in SOD, CAT and GSH activities. However, peel extract administration along with T_4 decreased hepatic LPO, serum glucose concentration and en-

Table 3 Effect of three different concentrations of test peel. Extract in the serum concentrations of glucose, T_3 and T_4 and on hepatic LPO. (n = 7)

Parameters	Control	HD (200 mg/kg)	MD (100 mg/kg)	LD (50 mg/kg)
T_3 (ng/ml)	0.79 \pm 0.03	0.69 \pm 0.05	0.33 \pm 0.01 a	0.81 \pm 0.13
T_4 (ng/ml)	53.40 \pm 2.40	59.50 \pm 3.36	29.14 \pm 1.62 a	57.57 \pm 2.69
LPO (nm MDA/h/mg protein)	0.51 \pm 0.07	0.45 \pm 0.07	0.29 \pm 0.04 c	0.59 \pm 0.06
Glucose (mg/dl)	101.84 \pm 0.34	94.40 \pm 3.00	73.80 \pm 5.39 c	101.78 \pm 8.97

Values are means \pm SEM.

a: $p < 0.001$ compared with the respective control values.

c: $p < 0.05$ compared with the respective control values.

Table 4 Effect of *Lagenaria siceraria* peel extract (PE) on the changes in hepatic protein, LPO, SOD, CAT, GSH and G-6 Pase; in serum T_3 , T_4 and glucose concentrations in female mice. (n = 7)

Parameters	Control	T_4	T_4 + PE (100 mg/kg)
T_3 (ng/ml)	0.06 \pm 0.04	1.80 \pm 0.04 a	0.66 \pm 0.29 x
T_4 (ng/ml)	35.71 \pm 2.02	60.00 \pm 4.96 b	22.12 \pm 2.90 x
LPO (nm MDA/h/mg protein)	0.51 \pm 0.06	1.29 \pm 0.26 c	0.70 \pm 0.16 x
SOD (units/mg protein)	5.27 \pm 0.27	2.32 \pm 0.31 a	8.66 \pm 0.57 x
CAT(μM H_2O_2 decomposed/mg protein)	38.81 \pm 1.64	19.03 \pm 0.51 a	32.40 \pm 1.59 x
GSH (μM GSH/mg protein)	2.50 \pm 0.15	0.54 \pm 0.07 a	3.38 \pm 0.32 x
G-6PASE (μM Pi liberated/min/mg protein)	0.18 \pm 0.01	0.27 \pm 0.01 a	0.14 \pm 0.02 x
GLUCOSE (mg/dl)	90.72 \pm 2.31	143.39 \pm 4.06 a	67.49 \pm 11.26 x

Values are means \pm SEM. a: $p < 0.001$, b: $p < 0.01$, c: $p < 0.05$ when compared with the control values and x: $p < 0.001$ when compared with T_4 -treated values.

T_4 : thyroxine-treated; T_4 + DRUG: thyroxine-treated + peel extract.

hanced the activity of SOD and CAT and the total GSH content significantly.

DISCUSSION

Results of the *in-vitro* study clearly revealed the free radical quenching potential of the peel extract at all the doses used, as evidenced by a decrease in DPPH free radicals. The test extract was also found to inhibit hepatic LPO, induced either by CCl_4 or by H_2O_2 , suggesting its antiperoxidative role. When the same peel extract was evaluated *in vivo*, it also exhibited antiperoxidative activity. However, out of three different doses (50, 100 and 200 mg/kg), only 100 mg/kg could inhibit hepatic LPO suggesting that this dose of *L. siceraria* peel extract may be considered as safe and also antiperoxidative. A somewhat similar dose-dependent effect in tissue LPO has also been observed earlier with respect to other vegetables such as *Momordica charantia*, *Moringa oleifera*, *Allium sativum* and *Trigonella foenum gracum* (Panda and Kar 2000; Tahiliani and Kar 2000, 2003a, 2003b). Interestingly, the antioxidative property of the test peel was also exhibited in hyperthyroid animals as it decreased the hepatic LPO induced by L-T₄ administration. There was also parallel increase in the activity of defensive enzymes such as SOD and CAT and also in GSH content, further supporting the antioxidative role of the peel extract. Although the whole fruit of *L. siceraria* was earlier reported to be antioxidative (Shirwaikar and Sreenivasan 1996), nothing was known on its peel extract, which is reported now. As in our study the test peel was found to be antiperoxidative, it appears to be useful for the regulation of free radical induced diseases. This holds true because of the fact that, antioxidants through their scavenging power are useful for the management of various diseases including cardiomyopathy, cancer and diabetes (Koleva *et al.* 2002).

With respect to the thyroid function and glucose metabolism, the peel extract inhibited the concentrations of both the thyroid hormones, T₄ and T₃ and also the serum glucose in euthyroid animals, particularly at 100 mg/kg, indicating that *L. siceraria* peel may serve as anti-thyroid and antihyperglycemic agent, when used in moderate amount. Interestingly the test peel could also ameliorate hyperthyroidism as well as hyperglycemia as it could decrease the T₄-induced elevation in serum thyroid hormones and glucose concentrations. A parallel decrease in hepatic glucose-6-phosphatase activity following the peel extract administration further supports the thyroid inhibitory role of the plant extract as the activity of this enzyme is directly related to its thyroid function (Ganong 1995). Although some fruit peel extracts have been reported to be antithyroidic in nature (Parmar and Kar 2007), the present one appears to be the first report on a vegetable peel that is capable of regulating thyroid dysfunctions.

Thyroid hormones are believed to be involved in the regulation of blood glucose metabolism and hyperthyroidism very often leads to hyperglycemia (Ganong 1999). In the present study an increase in blood glucose was observed following L-T₄ administration as reported earlier (Tahiliani and Kar 2003b; Parmar and Kar 2008). However, the thyroid hormone-induced increase in serum glucose was decreased by the administration of the test peel extract suggesting its potential to regulate hyperglycemia. This could be because of its active components such as flavonoids, sterols, cucurbitacin, saponins, and polyphenolics (Ghule *et al.* 2006b) that are known to inhibit glucose formation or through a decrease in glucose-6-phosphatase activity (as also observed in this study) that usually increases glucose synthesis.

In conclusion, our present findings reveal that the peel extract of *Lagenaria siceraria*, not only regulates lipid peroxidation, but also appears to be a promising agent for the amelioration of hyperthyroidism or hyperglycemia.

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