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Research Article

FICUS CARICA LEAF EXTRACT IN REGULATION OF THYROIDISM USING ELISA TECHNIQUE

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ABSTRACT

Materia Medica of India provides information on the folklore practices and traditional aspects of therapeutically important natural products. *Ficus carica* Linn. (Moraceae) commonly known as the fig plant was evaluated for its ameliorative effect in the regulation of thyroidism in rat model because one of the chemical constituent tyrosine mentioned in the text of Materia Medica, is responsible for formation of T_3 and T_4 hormones.

Male albino rats of 120-150 g were treated orally with doses of 500mg/kg; 250 mg/kg and 125 mg/ Kg of ethanolic extract of *Ficus carica* leaf. Propylthiouracil (PTU) (10 mg/kg;sc) and Thyroxine (T_4)(0.5 mg/kg;i.p.) were used as standards for anti thyroid and thyroid drug. The treatments were given between 9.00 and 10.00 h of the day to avoid circadian variation and continued for 21 days. On the day 8th, 15th and 22nd of the experiment, Twenty-four hours after the last dose, blood was collected by retro orbital route and serum samples were stored at -20^{0}C until assayed for T_3 and T_4.

L-T_4 administration (0.5 mg/kg/d for 21 days, i.p.) increased the levels of serum T_3 and T_4, However, simultaneous administration of the *Ficus carica* leaf extract (500 mg/kg, 250mg/kg, and 125mg/kg), effects indicating their potential in the regulation of thyroidism. The relative efficacy of plant extract was compared with propylthiouracil (PTU), a standard antithyroid drug and Thyroxine (T_4), a standard thyroid drug.

The relative potency of plant extract was calculated in terms of percent increase or decreases in thyroid hormones, to the control value, the increase in T_3 and T_4 concentration indicate its possible use in the regulation of hypothyroidism. Phytochemical analyses including HPTLC revealed the presence of tyrosine in the leaf extract which may be suggestive of thyroidal activity of *Ficus carica* leaf extract depending on the well established mechanism of T_3, T_4 formation in the body. The authors recommend further research work to fully explore its mechanism of its effect of T_3, T_4 formation in the body.

Key words: *Ficus carica*, thyroid, H.P.T.L.C., E.L.I.S.A

INTRODUCTION

Thyroid hormonal disorders are associated with the imbalance of T_3 and T_4 hormones secreted by the thyroid gland directly into the blood, the severity of thyroid hormonal imbalance leads to some of the common diseases like diabetes and hypertension and disturb the BMR of the body. Approximately half the cases of thyroid disease involve hyperthyroidism and the other half involves hypothyroidism. Despite the fact that day-by-day herbal drugs are gaining much importance for their affordable and safe nature, scientific investigations towards the mitigation of thyroid disorders by the plant extracts are meager. [1,2,3,4,5,6,7,8] In almost all of these reports, only one thyroid hormone (T_3 or T_4) was altered by the plant extract.[9,10] Therefore, in our endeavor to find out a plant extract that can regulate the levels of both the thyroid hormones,*Ficus carica* Linn. (Moraceae) commonly known as the fig plant, a native of Carica in Asia is grown in nearly all tropical and sub-tropical countries. It is now cultivated chiefly in the Mediterranean region, from Turkey in the east to Spain and Portugal in the west; it is also grown commercially in parts of U.S.A. and Chile and, to a small extent, in India, Arabia china and Japan.[11]The plant has several folkloric uses in many traditional system of medicines like mild laxative, expectorant, diuretic, good nutritional support for diabetics, aphrodisiac tonic and used in checking haemorrhage.[12,,13,14] India has an ancient heritage of traditional medicine. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products.[15] Compliance is a major problem with synthetic drugs as they have to be administered empty stomach, hence it was thought worthwhile to explore the available herbal option using ethanolic extract of *Ficus carica* leaves.

MATERIALS AND METHOD

Experimental Animals

Adult albino rats of male sex weighing between 120-150 g procured from our animal house were housed under standard environmental conditions in polypropylene cages (25±1°C temperature, 55±5% humidity and 12 h/12 h light/dark cycle). The animals were allowed free access to drinking water and rat feed. The care and handling of rats were in accordance with the protocol approved by Institutional Animal Ethics Committee (ref: BBDGEI/IAEC/04/2011).

Plant Material

The plant material was collected from local areas of Lucknow, Uttar Pradesh and was authenticated from National Botanical Research Institute, Lucknow by depositing a voucher specimen (Ref No. NBRI/CIF/178/2010) and was authenticated as *Ficus carica* L. (Moraceae)

Preparation of Extracts

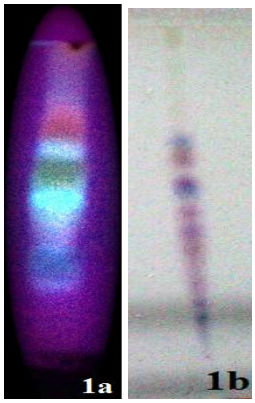
The leaves were washed thoroughly firstly with tap water and finally with double distilled water, shade dried at room temperature for more than two weeks and were powdered finely with mesh size 40 for extraction. The dried powdered drug (leaves) 200 g was successively Soxhlet extracted for a 72 hour cycle with n-hexane, ethanol and water and the percent yields was 15.28, 15.04, and 12.34 respectively.

Chemicals

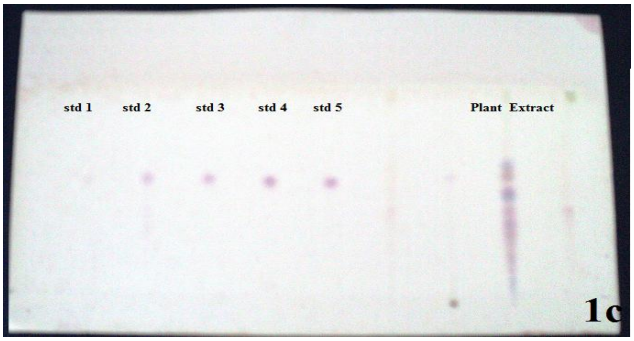
L-Thyroxine (T_4), Propylthiouracil (PTU) was procured from Sigma Chemical Co., USA. Enzyme linked immunosorbent assay (ELISA) kits for the estimation of total serum triiodothyronine (T_3) and T_4 were supplied by Weldon biotech (India) Pvt. Ltd., tyrosine, and glycerin.

Standardization of Plant Extract

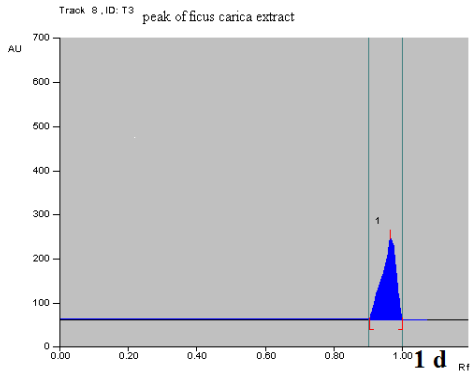
The ethanolic extract was subjected to phytochemical tests, which showed the presence of alkaloids, tannins and phenolic compounds, proteins, amino acids, steroids, terpenoids, and flavonoids which were confirmed by TLC. Chromatographic studies of amino acids was carried out using phenol-water(3:1) as mobile phase and 2% ninhydrin, and U.V.at long wavelength as detecting agents(fig 1a,1b).[16] Quantitative estimation of tyrosine was attempted with the help of HPTLC system equipped with a sample applicator device Camag Linomat 5. Camag twin trough chamber, Camag TLC scanner and integration software (Wincats). Increasing serial dilutions of tyrosine working standards (200-1000 µg mL^{-1}) along with the test extract were scanned at 366 nm (Mobile phase: phenol-water (3:1)) to ascertain the amount of tyrosine present in the test extract(fig 1c,1d,1e, 1f). The estimated value was found to be 921.0ug/ml.



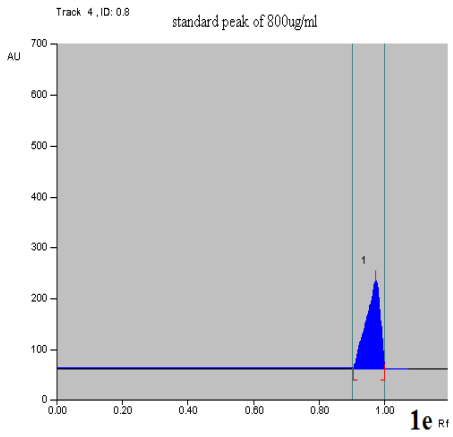
1a: t.l.c of plant extract for amino acids at u.v; 1b: t.l.c of plant extract for amino acids after spraying 2 %ninhydrin.



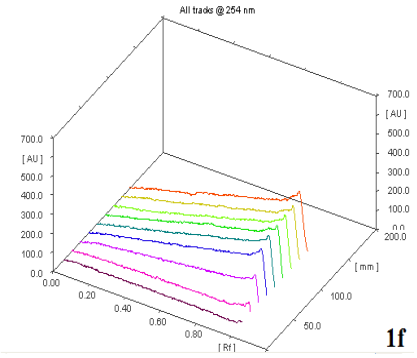
1c: precoted plate of h.p.t.l.c. with 5 differentconcentrations of standard tyrosine and plant extract after spraying 2% ninhydrin.



1d: Peak of *ficus carica* extract at 254nm.



1e: Peak of 4th concentration of standard tyrosine at 254nm.



1f:3d graph with 5 different concentrations of standard tyrosine and plant extract at 254nm.

Acute Toxicity Studies

The acute toxicity studies were performed in accordance with the OECD (Organization for Economic Co-operation and Development) guidelines no. 425 (Up and Down Procedure). No death was observed till the end of the study. The test samples were found safe up to the dose of 2000mg/kg and from the results 500 mg/kg was chosen as the maximum dose for further experimentation. [17]

Experimental design:

The rats were divided randomly into six groups of seven each. While the animals of groups IV- VI were administered with the plant extract by gastric incubation at the doses of 500.0 mg/kg ; 250 mg/kg and 125 mg/kg respectively, groups II and III were treated with PTU (10 mg/kg) and T_4 (0.5 mg/kg), respectively. The animals of group I, receiving an equal amount of vehicle alone served as control. All the treatments were given between 9.00 and 10.00 h of the day to avoid circadian variation and were continued for 21 days. [18] On the day 8th, 15th and 22nd of the experiment, twenty-four hours after the last dose, blood was collected by retro orbital route and serum samples were stored at -20^{0}C until assayed for T_3 and T_4. [8]

Estimation of thyroid hormones

Serum concentrations of total T_3 and T_4 were estimated by ELISA [19]as per the routine protocol followed in our laboratory. Lower limits of sensitivity for T_3 and T_4 were 0.04ng/dl and 0.4µg/dl and respectively. Inter-assay variation was less than 5% for both hormones. [20, 21, 22, 23]

Statistical Analysis:

All results were expressed as mean ± standard error of mean (S.E.M.). Data was analyzed using one-way ANOVA followed by Dunnett's-test. $P < 0.05$ was considered as statistically significant.

RESULTS

T_3 concentration and T_4 concentration in ng/ml for 07 days:

A significant increase in serum T_4 concentration (Table 1) was observed in all the groups when compared to T_3 concentration. As compared to the control value, T_3 and T_4 concentration increased in the all the groups except III group which is clearly shown by the percentage change in hormone concentration. Thyroid hormone concentrations were decreased in PTU and increased in T_4 treated groups (positive for the entire group except III group). The group which received maximum test dose (500mg/kg) showed maximum percentage increase in hormone concentration when compared to the other two dose level which clearly proves that the response was dose effective and can be used in hypothyroidism condition to normalize hormone level.

T_3 concentration and T_4 concentration in ng/ml for 14 days

The order of response in 14 days observation was same as that of 07 days but the percentage change in hormone concentration slightly increased. A significant increase in serum T_4 concentration (Table 2)

was observed in all the groups when compared to T_3 concentration which was in the range of 10.89±0.27 to53.58±0.26 and0.520±0.36 to 1.335±0.25 respectively. As compared to the control value, T_3 and T_4 concentration increased in the all the groups except III group which is clearly shown by the percentage change in hormone concentration of-24.23 and -17.90 for T_3 and T_4 respectively and all the other values were in positive. The group which received maximum test dose (500mg/kg) showed maximum percentage increase in T_3 hormone concentration (+103.07) when compared to the other two other dose level and in case ofT_4 hormone concentration the lower dose (125mg/kg) showed the maximum response (+269.05) which was slightly more than the high dose (500mg/kg) which produce +249.21 value which shows that the response was effective in terms of concentration and time, might be suggestive in hypothyroidism condition.

Table 1: t_3 concentration and t_4 concentration in ng/ml for 07 days along with their percent increase (+) or decrease (-) in relation to the control values in male rats.

Groups	T_3 concentration ng/ml	T_4 concentration ng/ml	percent change in T_3 concentration	percent change in T_4 concentration
I-(Control)	0.428±0.23[a]	11.25±0.31[b]	-	-
II-(T_4)	1.302±0.35	48.35±0.21	+204.2	+303.11
III-(PTU)	0.404±0.65	9.08±0.36	-4.90	-19.28
IV-FCEC (500mg/kg)	0.595±0.21[a1]	27.71±0.31[b1]	+39.02	+146.31
V-FCEC (250mg/kg)	0.551±0.45[a2]	27.39±0.24[b2]	+28.37	+143.46
VI -FCEC (125mg/kg)	0.437±0.15[a3]	22.89±0.14[b3]	+2.10	+103.46

Data are means±S.E.M. (n = 7). a,b($P < 0.05$ as compared to positive control using one way anova) a1,a2,b1,b2,b3($P < 0.01$), a3($P > 0.05$**)** as compared to the respective control values using Dunnett's Multiple Comparison Test. FCEC – *Ficus carica* ethanolic extract

FCEC- Ficus carica ethanolic extract**, PTU-** Propylthiouracil

Table 2: t_3 concentration and t_4 concentration in ng/ml for 14 days along with their percent increase (+) or decrease (-) in relation to the control values in male rats:

Groups	T_3 concentration ng/ml	T_4 concentration ng/ml	percent change in T_3 concentration	percent change in T_4 concentration
I-(Control)	0.520±0.36[a]	10.89±0.27[b]	-	-
II-(T_4)	1.335±0.25	53.58±0.26	+156.73	+392.01
III-(PTU)	0.394±0.34	8.94±0.34	-24.23	-17.90
IV-FCEC (500mg/kg)	1.056±0.31[a1]	38.03±0.54[b1]	+103.07	+249.21
V-FCEC (250mg/kg)	0.830±0.28[a2]	32.27±0.16[b2]	+59.61	+196.32
VI -FCEC (125mg/kg)	0.954±0.14[a3]	40.19±0.15[b3]	+83.46	+269.05

Data are means±S.E.M. (n = 7). a,b($P < 0.05$ as compared to positive control using one way anova) a1,a2, a3,b1,b2,b3($P < 0.01$) as compared to the respective control values using Dunnett's Multiple Comparison Test.

FCEC- Ficus carica ethanolic extract**, PTU-** Propylthiouracil

T_3 concentration and T_4 concentration in ng/ml for 21days

The administration of Propylthiouracil (PTU, a thyroid function inhibitor) at a dose of 10 mg/kg/day i.p. for 21 days to rats decreased the serum concentration of both the thyroid hormones (T_3 andT_4) (table:3). From the observation it was evident that 0.5 mg L-Thyroxine treatment for 21 days significantly increased the serum T_3 and T_4 concentrations. With a concomitant increase in serum levels of both the thyroid hormones (T_3 and T_4) were observed in experiment after 21 days in case of test dose dependent groups as compared to the control group. The administration of Propylthiouracil (PTU, a thyroid function inhibitor) at a dose of 10 mg/kg/day i.p. for 21 days to rats decreased the serum

Table 3: t_3 concentrations and t_4 concentration in ng/ml for 21days along with their percent increase (+) or decrease (-) in relation to the control values in male rats:

Groups	T_3 concentration ng/ml	T_4 concentration ng/ml	percent change in T_3 concentration	percent change in T_4 concentration
I-(Control)	0.589±0.15[a]	10.28±0.32[b]	-	-
II-(T_4)	1.3765±0.21	71.55±0.12	+133.61	+596.01
III-(PTU)	0.378±0.36	8.47±0.16	-35.82	-17.60
IV-FCEC (500mg/kg)	1.434±0.24[a1]	57.20±0.24[b1]	+143.52	+456.42
V-FCEC (250mg/kg)	1.248±0.21[a2]	36.38±0.27[b2]	+111.88	+253.89
VI-FCEC (125mg/kg)	1.063±0.18[a3]	47.77±0.31[b3]	+80.47	+364.68

Data are means±S.E.M. (n = 7). a,b($P < 0.05$ as compared to positive control using one way anova) a1,a2, a3,b1,b2,b3($P < 0.01$) as compared to the respective control values using Dunnett's Multiple Comparison Test.

FCEC- Ficus carica ethanolic extract**, PTU-** Propylthiouracil

Concentration of both the thyroid hormones (T_3 andT_4). From the observation it was evident that 0.5 mg L-Thyroxine treatment for 21 days significantly increased the serum T_3 and T_4 concentrations. The percentage change in T_3 concentration was maximum in case of high dose (500mg/kg) as compared to the Thyroxine group i.e. +143.52 and+133.61 respectively, whereas it was vice versa for percentage change in T_4 concentration i.e. +596.01 and +456.42 respectively.

DISCUSSION

T_3 concentration ng/ml

T_3, which is considered as the most metabolically active hormone, is synthesized in lesser amount by the gland and is formed mostly in liver by monodeiodination of T_4. [18] Thus the syntheses or formation (concentration) of T_3 increases from day7 to day 14 to day21 as seen from the observation (table: 4) for all the groups except the PTU group. The concentration was found to be maximum in case of last observation of high dose. On comparing the results from control group the concentration was increased from low dose to medium to high dose and from day 7 to day 21(fig 2a).

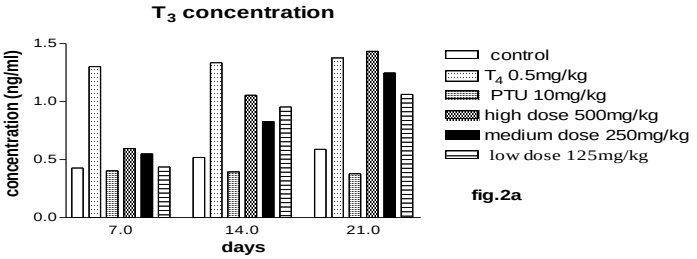


Fig 2: A- Concentration of T_3 Hormone in 7, 14, 21 Days,

Table 4: t_3 concentrations in ng/ml

Groups	T_3 concentration (ng/ml) in 7 days	T_3 concentration (ng/ml) in 14 days	T_3 concentration (ng/ml) in 21 days
I-(Control)	0.428±0.23[a]	0.520±0.36[a]	0.589±0.15[a]
II-(T_4)	1.302±0.35[b]	1.335±0.25[b]	1.3765±0.21[b]
III-(PTU)	0.404±0.65[c]	0.394±0.34[c]	0.378±0.36[c]
IV-FCEC (500mg/kg)	0.595±0.21[d]	1.056±0.31[d]	1.434±0.24[d]
V-FCEC (250mg/kg)	0.551±0.45[e]	0.830±0.28[e]	1.248±0.21[e]
VI -FCEC (125mg/kg)	0.437±0.15[f]	0.954±0.14[f]	1.063±0.18[f]

Data are means±S.E.M. (n = 7). Effects of PTU and T4 have also been included and found to be significantly different at P < 0.05 as compared to positive control using one way anova, b(P < 0.01), c,d,e,f(P > 0.05**)** as compared to the respective control values using Dunnett's Multiple Comparison Test.

FCEC- Ficus carica ethanolic extract**, PTU-** Propylthiouracil

T_4 concentration in (ng/ml)

The observation regarding the concentration of T_4 was same as that of T_3 i.e. time effective and dose effective but concentration value was high enough as compared to T_3(fig 2b). Propylthiouracil (PTU), a thyroid function inhibitor decreased the serum concentration of both the thyroid hormones (T_3 andT_4) table 5, whereas L-Thyroxine treatment significantly increased the serum T_3 and T_4 concentrations. Increase in concentration of T_4 from day7 to day14 to 21day in the entire dose level stimulant to thyroid functions.

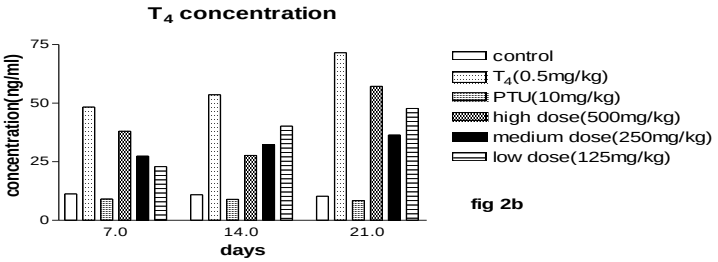


Fig 3: B- Concentration of T_4 Hormone in 7, 14, 21 Days.

Table 5: T_4 Concentrations in Ng/Ml

Groups	T_4 concentration (ng/ml) in **7 days**	T_4 concentration (ng/ml) in **14 days**	T_4 concentration (ng/ml) in **21 days**
I-(Control)	11.25±0.31[a]	10.89±0.27[a]	10.28±0.32[a]
II-(T_4)	48.35±0.21[b]	53.58±0.26[b]	71.55±0.12[b]
III-(PTU)	9.08±0.36[c]	8.94±0.34[c]	8.47±0.16[c]
IV-FCEC (500mg/kg)	27.71±0.31[d]	38.03±0.54[d]	57.20±0.24[d]
V-FCEC (250mg/kg)	27.39±0.24[e]	32.27±0.16[e]	36.38±0.27[e]
VI -FCEC (125mg/kg)	22.89±0.14[f]	40.19±0.15[f]	47.77±0.31[f]

Data are means±S.E.M. (n = 7). Effects of PTU and T_4 have also been included and found to be significantly different at P < 0.05 as compared to positive control using one way anova, b,d(P < 0.01), c,e,(P > 0.05**),** f(P<0.05) as compared to the respective control values using Dunnett's Multiple Comparison Test

FCEC- Ficus carica ethanolic extract**, PTU-** Propylthiouracil

CONCLUSION

The prevalent circulating thyroid hormone is T_4 which is secreted from the thyroid gland, whereas T_3, which is considered as the most metabolically active hormone, is synthesized in lesser amount by the gland and is formed mostly in liver by monodeiodination of T_4.[18] As T_4 is synthesized only in thyroid gland and all the three doses of the plant extract increased the concentrations of this hormone, it appears that the leaf extract induced increase in T_4 concentration could either be the result of direct stimulation of T_4 synthesis and/or release at the thyroidal level. However, increase in serum T_3 concentration by all the three doses could be due to the stimulation in monodeiodination of T_4 in peripheral tissues, which is known to be the major process of its synthesis. Another possibility of plant extract-induced increase in thyroid hormone concentrations could be the decreased utilization of the hormones by the body. Although further investigations are required to reveal the exact mechanism of action(s) of thyroregulatory role of *Ficus carica* leaf extract, the present findings clearly indicate that this extract is stimulant to thyroid functions. However, the authors emphasize that further studies are required to observe the dose dependant effect of leaf extract which might be effective and safe in ameliorating hypothyroidism.

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