

Synergistic interactions of *Aegle marmelos* leaf, *Emblica officinalis* fruit and *Ocimum sanctum* leaf extracts in the regulation of hyperthyroidism and / or hyperglycaemia

Sunanda Panda and Anand Kar*

Thyroid research unit, School of Life Sciences, Devi Ahilya University, Vigyan Bhawan, Khandwa Road, Indore-452017, India

SUMMARY

The effects of *Aegle marmelos* (Rutaceae) leaf, *Emblica officinalis* (Euphorbiaceae) fruit and *Ocimum sanctum*. (Labiatae) leaf extracts were studied in L-thyroxine (0.5 mg/kg) induced hyperthyroidic mice. Separately combined effects of these three plant extracts and of a commonly used antithyroidic drug, Propyl thiouracil (PTU) were investigated for comparison. Serum concentration of thyroxine (T₄), triiodothyronine (T₃), glucose and the activity of hepatic Glucose 6-Phosphatase (G-6-Pase) were considered as main parameters. Hepatic lipid peroxidation (LPO), superoxide dismutase (SOD) and Catalase (CAT) activities were also studied to reveal the toxic effect of the plant extracts, if any. While exogenous T₄ enhanced serum concentration of T₄, T₃, glucose and the activity of hepatic G-6-Pase, a simultaneous administration of either *A. marmelos* leaf (1.0 mg/kg), *E. officinalis* fruit (30 mg/kg) and *O. sanctum* leaf (50 mg/kg) extracts, to hyperthyroidic animals decreased all these parameters. However, the effects were more pronounced, as nearly normal thyroid function and serum glucose concentration were exhibited when all three plant extracts were administered together. A decrease in LPO and a concomitant increase in SOD and the CAT activities indicated the safe and antiperoxidative nature of the plant extracts, administered either alone or in combination. Our findings reveal that the three test plant materials exhibit synergistic effects without any hepatotoxicity, suggesting their potential use in the amelioration of hyperthyroidism and/ or hyperglycaemia.

Key words: *Aegle marmelos*, *Emblica officinalis*, *Ocimum sanctum*; Synergistic effects; Hyperthyroidism; Hyperglycaemia

INTRODUCTION

Thyroid hormones are known to regulate almost all body functions either directly or indirectly (Ganong, 1995). Therefore, deficiency (hypothyroidism) /over secretion (hyperthyroidism) of two thyroid hormones, thyroxine (T₄) & triiodothyronine (T₃) leads to several health problems. Particularly hyperthyroidism, if not treated for a long time, very often ends up with clinical abnormalities including hyperglycaemia, some times referred as

thyroid diabetes (Roith *et al.*, 1996).

Although some allopathic medicines including Propyl thiouracil & Neomarkazole are available for the treatment of hyperthyroidism, these drugs normally exhibit side effects, such as myxedema and agranulocytosis. As herbal medicines are considered to be safe as well as effective, in the present investigation an attempt has been made to regulate hyperthyroidism with the use of three plant extracts administered either alone or in combination.

Despite the fact that the synergistic effects of two or more plant extracts are commonly considered to be additive & physiologically more potential than the individual plant extract (Williamson, 2001), reports on the combined effects of plant extracts in

*Correspondence: Dr Anand Kar, Thyroid Research Lab, School of Life Sciences, Devi Ahilya University, Takshashila Campus, Khandwa Road, INDORE- 452017, India. Tel: +091-0731-477166; Fax: +091-0731-2460830; E-mail: karlife@rediffmail.com

the regulation of hyperthyroidism are meager (Kar and Panda, 2003; Tahiliani & Kar, 2003b). Therefore in the present investigation effort has been made to reveal the combined effects of three different plant extracts, if any, in the regulation of hyperthyroidism in mice.

Aegle marmelos Linn. (Rutaceae), *Emblica officinalis* Gaertn. (Euphorbiaceae) and *Ocimum sanctum* Linn. (Labiatae) were considered as experimental plants because of the fact that all three plants were earlier found to inhibit thyroid functions in euthyroid laboratory animals (Panda & Kar, 1998; Panda *et al.*, 2002; Kar *et al.*, 2002). However, it was not known, whether they are effective in the regulation of hyperthyroidism. Therefore, the present investigation was made in T₄ induced hyperthyroid animals. Simultaneously effects of the individual plant extracts and of a commonly used antithyroidic drug, Propyl thiouracil (PTU) were also studied for a comparison. Serum concentration of T₄, T₃, glucose and the activity of hepatic glucose 6-Phosphatase (G-6-Pase) were considered as main parameters. Lipid peroxidation (LPO), superoxide dismutase (SOD) and Catalase (CAT) activities were also studied in liver (the primary target organ of a drug) to reveal the toxic effects of the plant extracts, if any.

MATERIALS AND METHODS

Plant materials

Emblica officinalis (Euphorbiaceae) fruits, *Aegle marmelos* (Rutaceae) leaf and *Ocimum sanctum* (Labiatae) leaf were collected locally, identified and the voucher specimens (No. E: 112, A:101 and O:115 respectively) were deposited in the departmental herbarium for future reference.

Preparation of the extracts:

A. marmelos extract (AM) was prepared according to the method of Rao *et al.*, (1995). In brief, fresh leaves were dried under shade and then powdered. Leaf powder (200 g) was mixed with 500 ml distilled water and boiled. After filtration through Whatman filter paper # 40, extract was dried by slow heating and continuous stirring. The yield of the extract was 20%. The leaf extract was dissolved in distilled water (d.w.) and administered

orally at a dose of 1 g /kg. (Seema *et al.*, 1996).

E. officinalis (EO) fruits of good quality were air-dried and then ground in to fine powder by pulverization. The dried powder was extracted with 95% ethanol at 60°C by soxhlation as described earlier (Panda *et al.*, 2002). The extract, (approximately 12% of air dried powder) dissolved in d. w. was orally administered at a dose of 30 mg/kg.

Fresh leaves of *O. sanctum* (OS) of dark leaved variety were collected locally and were shade dried. Refluxing the powdered leaves with distilled water performed water extraction and then the extract was vacuum dried. (Panda and Kar, 1998). The yield of this extract was found to be 12% of the air-dried powder. The extracts were dissolved in double distilled water for oral administration and were administered at the dose of 50 mg/kg (Sharma *et al.*, 2002).

Animals

Adult healthy colony breed Swiss male albino mice (30±2 g) were used for experimentation. The animals were maintained under standard condition of housing (27±1°C; 14: 10 Light dark cycle) in polypropylene cages with the provision of mice feed (Golden feeds, New Delhi, India) and water *and libitum*.

Experimental design

Experiment 1. Out of forty male mice, thirty-two were rendered hyperthyroidic by daily subcutaneous injection of L-T₄ at a dose of 0.5 mg/kg./d for 12 consecutive days as used earlier (Panda and Kar, 2000, 2001, 2003). They were then divided into five groups of eight each. While gr. II continued to receive only T₄, gr. III, IV, and V, were treated with equivalent dose of T₄ along with EO (30 mg/kg/d), OS (50 mg/kg/d) and AM (1.0g/kg/d) extracts respectively everyday for fifteen days, while, gr. I animals received only 0.1 ml of vehicle and served as control.

Experiment 2. Thirty-two animals were divided into four groups of 8 each. Twenty four (group II-IV) were rendered hyperthyroidic by daily subcutaneous injection of L-T₄ at a dose of 0.50 mg /kg./d for 12 consecutive days and were then divided in to three groups of eight each. Group II continued to receive only T₄, while gr. III was

treated with equivalent dose of T_4 along with EO (30 mg/kg/d), AM (1.0 g/kg/d) & OS (50 mg/kg) extracts, all together. Group IV was administered (i.p) with Propyl thiouracil (PTU) at a pre-standardized dose of 10 mg/kg/d (Kar *et al.*, 2002) along with T_4 . Gr. I animals received only vehicle and served as control (CRL).

All the doses were administered between 10.00 and 11.00 hr of the day to avoid circadian variations and both the experiments were continued for 15 days.

Serum collection and biochemical estimations

Twenty-four hours after the administration of the last dose, animals were sacrificed by cervical dislocation. Blood from each one was collected and serum samples were stored at -80°C until assayed for total T_3 and T_4 by radioimmunoassay (RIA). Liver was removed quickly, washed thoroughly with phosphate buffered saline (PBS, pH 7.4) and processed for biochemical estimations. LPO was estimated by thiobarbituric acid (TBA) reaction with Malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids, according to the method of (Ohkawa *et al.*, 1979) as modified by Jamall and Smith (1985). LPO was expressed as nM of MDA formed/hr/mg protein. Hepatic SOD activity was assayed according to the method of Marklund and Marklund (1974). The enzyme activity was expressed as units/mg protein and one unit of the enzyme is defined as the activity that inhibits autoxidation of pyrogallol by 50%. Catalase (CAT) activity was estimated following the method of Aebi (1983). For the determination of serum glucose, the modified glucose oxidase method of Hugget and Nixon (1957) was followed using GOD-POD kits from Sigma diagnostic Ltd, Baroda, India. Hepatic G-6-Pase activity was studied by the method of Baginski *et al.* (1974).

Estimation of thyroid hormones and protein:

For the assay of thyroid hormones RIA kits were used, procured from Bhabha Atomic Research Center, Mumbai, India and serum concentrations of total T_3 and T_4 were estimated using routine RIA protocols followed earlier in our laboratory (Panda & Kar, 1999, 2001). In brief RIA was performed using Tris hydroxymethyl amino methane buffer

(0.14 M; pH 8.6). The reaction mixture comprised of standard /sample, buffer, ^{125}I - T_4 / T_3 antibody. The tubes were incubated at 37°C for 30 min (for T_4)/45 min (for T_3). Centrifugation was at 2000 g for 20 minutes. For radioactivity counting ^{125}I gamma counter was used. Quality controls were also run simultaneously. Lower limits of sensitivity for T_3 and T_4 were 0.07 ng/ml and 0.12 ng/ml respectively. Inter-assay variation was less than 5% for both the hormones.

The hepatic protein content was determined using bovine serum albumin as standard by the method of Lowry *et al.* (1951).

Statistical analysis

Data are expressed as the mean \pm standard error of the mean (SEM). For statistical evaluation, analysis of variance (ANOVA) followed by *Student's t*-test was considered. Statistical significance was ascribed only when p-value was less than 0.05.

RESULTS

Experiment-1. Administration of L- T_4 to normal (euthyroid) mice increased the serum concentrations of T_3 , T_4 , glucose, & the hepatic G-6-Pase activity significantly ($P < 0.001$) when compared to the respective control values (Fig. 1).

The oral administration of AM/EO/OS extract in the T_4 -induced mice significantly decreased the concentration of serum glucose, T_3 and T_4 , ($P < 0.001$ for all) with a parallel decrease in hepatic G-6-Pase activity ($P < 0.05$, 0.01, 0.001 respectively) as compared to the values of the group treated with T_4 only. While an increase in LPO & decrease in SOD & CAT activities was observed in T_4 treated group ($P < 0.001$ for all), reverse observations were made with respect to LPO ($P < 0.01$, 0.001 and 0.001 respectively), SOD ($P < 0.05$, 0.01 and 0.001 respectively) and CAT ($P < 0.001$, for all) activities following the administration of AM/EO/OS extracts along with T_4 .

Experiment 2. Here also T_4 administration enhanced the thyroid hormone concentrations, & G-6-Pase activity significantly ($P < 0.001$, for all). However, serum T_3 , T_4 & glucose concentration were decreased significantly by *Ocimum*+ *Aegle*+ *Embolica* extracts (P , < 0.001) as compared to T_4 treated values.

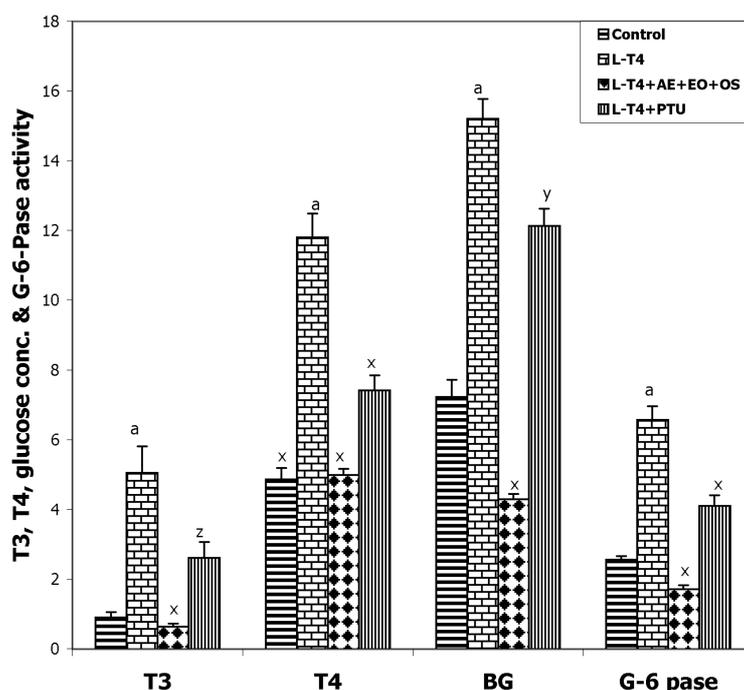


Fig. 1. Changes in serum concentrations of T₃ (ng/ml), T₄ (ng/ml ×10) and glucose (mg%) and hepatic G-6-Pase activity (μM phosphate generated/min/mg protein ×10⁻¹) following the administration of *A. marmelos* (AM)+*E. officinalis* (EO)+*O. sanctum* (OS) extracts or PTU for 15 days in hyperthyroid male mice. Each bar represents the mean ± SEM (n=8). T₄, thyroxine; T₃, Triiodothyronine; G-6-Pase, Glucose 6- phosphatase. ^aP<0.001, ^bP<0.01 and ^cP<0.05 as correspond to their respective control values. ^xP<0.001, ^yP<0.01 and ^zP<0.05 as compared to the respective T₄ treated values.

With respect to LPO, SOD and CAT activities, in these hyperthyroidic animals, receiving three plant extracts, hepatic LPO decreased, while SOD & CAT activities were enhanced significantly ($P<0.001$).

Administration of PTU along with T₄ decreased the concentrations of T₄, T₃, serum glucose and

Table 1. Effects of *Aegle marmelos* leaf (AM, 1.00 g/g), *Emblca officinalis* fruit (EO, 30.00 mg/kg) and *Ocimum sanctum* (OS, 50.00 mg/kg) leaf extracts on hepatic LPO (nM MDA formed/h/mg protein), SOD (units/mg protein) and CAT (μM H₂O₂ decomposed/min/mg protein) activities in L-T₄ induced hyperthyroid male mice

Groups	LPO	SOD	CAT
Control	0.87±0.04	6.85±0.40	41.57±1.41
L-T ₄	1.81 ^a ±0.24	2.38 ^a ±0.27	26.84 ^a ±0.94
L-T ₄ +AM	0.91 ^y ±0.10	5.05 ^z ±0.98	54.76 ^x ±3.29
L-T ₄ +EO	0.47 ^x ±0.03	4.92 ^y ±0.67	48.34 ^x ±3.16
L-T ₄ +OS	0.84 ^x ±0.07	6.42 ^x ±0.31	43.54 ^x ±1.91

Values are mean±SEM. ^aP<0.001 compared with the respective control values; ^xP<0.001; ^yP<0.01; ^zP<0.05 compared with the respective values of T₄ treated group.

G-6-Pase activity significantly ($P<0.001$, 0.05, 0.01 and 0.001 respectively) as compared to their corresponding values in T₄ treated groups. LPO & SOD activities remained unchanged, whereas, CAT activity was found to be increased significantly ($P<0.01$) in this group. When percent inhibition was calculated out, maximum inhibition in T₃ and in serum glucose concentration was found in the group that received all three plant extracts together (Table 3).

DISCUSSION

Results reveal that *A. marmelos*, *E. officinalis* and *O. sanctum* extracts, when administered individually, reduced serum T₃, T₄ & glucose concentrations as well as hepatic G-6-Pase activity in T₄ induced hyperthyroidic animals, as observed earlier with some other plant extracts (Panda & Kar, 2001, 2003; Tahiliani & Kar, 2003a). Interestingly, combined effects of these plant extracts (results of experiment 2) for most of the parameters studied, were much

Table 2. Combined effects(L-T₄+AM+EO+OS) of *A. marmelos* leaf, *E. officinalis* fruit and *O. sanctum* leaf extracts or Propyl thiouracil (PTU) on hepatic LPO (nM MDA formed/h/mg protein), SOD (units/mg protein) and CAT ($\mu\text{M H}_2\text{O}_2$ decomposed/min/mg protein) activities in L-T₄ induced male hyperthyroid mice

GROUPS	LPO	SOD	CAT
CONTROL	0.96±0.06	5.42 ±0.27	65.86±3.00
L-T ₄	1.98 ^a ±0.19	3.99 ^a ±0.32	44.04 ^a ±2.31
L-T ₄ +AM+EO+OS	0.55 ^x ±0.13	8.22 ^x ±0.47	82.09 ^x ±6.25
T ₄ +PTU	1.26 ±0.25	5.65 ±0.71	66.23 ^y ±7.15

Values are mean±SEM. ^aP<0.001 compared with the respective control values.

^xP<0.001; ^yP<0.01 compared with the respective values of T₄ treated group.

Table 3. Percent (%) decrease on serum of T₃, T₄ and glucose concentrations following the administration of AM, EO, and OS extracts either alone or in combination in relation to the values of L-T₄ treated hyperthyroid male mice

Groups	T ₃	T ₄	glucose
L-T ₄ +AM	87.30%	49.05%	35.91%
L-T ₄ +EO	61.64%	63.87%	27.19%
L-T ₄ +OS	80.68%	44.14%	59.88%
L-T ₄ +AM+EO+OS	87.50%	58.00 %	71.80%

more greater than that of individual extract. Although percent reduction in serum T₄ was little more (63%) when *Emblica* extract was administered alone, maximum decrease in T₃ (the metabolically most active thyroid hormone) & in serum glucose concentrations was observed by the administration of all three different plant extracts together. In fact, thyroid function and serum glucose level were nearly normalized in these animals suggesting that these three plant extracts in combination may potentially regulate thyroid diabetes/hyperthyroidism synergistically.

Despite a common belief that the therapeutic efficacy of mixture of herbs is more than the individual plant extract (Williamson, 2001) and the fact that some reports are already available on the effects of some herbal formulations/compounds on other disorders (Mirsalis *et al*, 1993, Ahmed and Sharma, 1997; Pari and Sarravaan, 2002), no investigation was made till to date on the combined effects of three plant extracts in the regulation of thyroxine induced hyperthyroidism /or hyperglycaemia. Therefore the present finding is quite significant to suggest the possible synergistic effects of three different plant extracts in the regulation of hyperthyroidism and /or hyperglycaemia.

When the combined effects were compared with

that of a standard antithyroidic drug PTU, the former was found to be more inhibitory to T₃, T₄ & glucose concentrations (87.50, 58.00 & 30.83% respectively) as compared to the latter (48.31, 40.70 & 30.83 % respectively) which further indicate that the said plant extracts in combination may prove to be more effective in regulating hyperthyroidism as compared to the traditional drug, PTU.

It is now well understood that about 90% of the serum T₃ is produced by the extrathyroidal conversion of T₄ in peripheral organs, particularly in liver and kidney (Visser *et al*, 1978). Therefore, a decrease in T₃ concentrations by individual plant extract or in combination could be due to their efficacy in inhibiting the monodeiodination of T₄, as suggested earlier for some other plant extracts (Panda & Kar, 2001, 2003). On the other hand, as the second thyroid hormone, T₄ is primarily produced in the gland itself, a decrease in serum T₄ concentrations following the administration of the plant extracts could be the result of a direct inhibition of T₄ synthesis & /or release at the glandular level. Another possibility in decrease in T₄ concentration could be an indirect effect through an inhibition in the production of thyroid stimulating hormone and /or thyrotropin releasing factor. Whatever may be the mechanism of action, our findings certainly suggest the potential synergistic effects of the three test plant extracts in regulating thyroid diabetes and thyrotoxicosis.

Hyperthyroidism is commonly associated with the disturbances in carbohydrate metabolism that results in impaired glucose tolerance, increase in peripheral insulin resistance & transient hyperglycaemia (Gorska *et al*, 1989; Ardawi and Khoja, 1993). This condition persists till the excess thyroid hormone is normalized. Interestingly, the three different plant

extracts in combination were able to normalize the thyroid hormones and glucose concentrations in thyroxine induced hyperglycaemic animals, suggesting that the experimental plant materials may have the potency to normalize the thyroid function synergistically.

Lipid peroxidation (LPO), a free radical chain reaction is the most common manifestation of tissue toxicity to various chemicals (Halliwell and Gutteridge, 1989). It is primarily an outcome of oxidations & formation of free radicals by peroxides & superoxides, which are generated continuously in living cells exposed to physiological stress and in this process, LPO alters the normal structural & functional properties of the cell, ultimately leading to cyto-toxicity and dismantling of the membrane structure (Kreps *et al*; 1986). The toxic lipid peroxides accumulated in the system are generally metabolized by cytosolic enzymes such as SOD & CAT to prevent any damage to biological membranes & to maintain cellular integrity (Seiss, 1993). An increase in LPO in response to various disease & tissue damage is maximally observed in liver cells, which are more susceptible to stress induced damage (Emerit & Chaudire, 1989) and the fact that liver is the common target organ of a drug, LPO activity of liver is always necessary for evaluating the possible toxic effect of a new drug. In the present investigation, exogenous thyroxine induced the level of hepatic LPO with a decrease in the activities of SOD & CAT. However, none of the plant extracts were found to increase LPO when administered either alone or in combinations, in T₄ treated animals suggesting that these plant extracts at the doses used, are apparently not toxic.

CONCLUSIONS

Prolonged hyperthyroidism is very often considered to be a cause for hyperglycaemia and diabetes mellitus. Our findings clearly indicate that the synergistic effects of *A. marmelos*, *E. officinalis* and *O. sanctum* extracts are more pronounced as compared to a standard antithyroid drug, PTU and to the individual extract. As investigated plant extracts were also found to be antiperoxidative in nature, their use for the amelioration of hyperthyroidism and / or hyperglycaemia may be considered. However, further investigation is a

must for optimization of the dose and duration for human therapy.

ACKNOWLEDGEMENTS

Financial assistance to Dr. (Ms) S. Panda (Research Associate) from Council of Scientific & Industrial Research (CSIR), New Delhi, India is gratefully acknowledged. We also acknowledge the facilities provided to us by the head of the department.

REFERENCES

- Aebi H. (1983) Catalase. In: *Methods in enzymatic analysis*, edited by Bergmeyer, HU, 3, p. 276, Academic press, New York.
- Ahmed RS, Sharma SB. (1997) Biochemical studies on combined effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale* Rosc.) in albino rats. *Ind. J. Exp. Biol.* 35, 841-843.
- Ardawi MS, Khoja SM. (1993) Effects of hyperthyroidism on glucose, glutamine and ketone body metabolism in the gut of the rat. *Int. J. Biochem.* 25, 619-624.
- Baginski ES, Fod PP, Zak B. (1974) Enzyme activities. In: *Methods in Enzymatic Analysis*, edited by Bergmeyer HU, 2, p. 876, Academic Press, New York.
- Emerit J, Chaudiere J. (1989) Free radicals and lipid peroxidation in cell pathology. In: *Hand book of free radicals and antioxidants in Biomedicine*, edited by Miquel J, Quintanilha AT, Weber H, p. 177, CRC press, Boca Raton, Florida.
- Ganong WF. (1995) *Review of Medical physiology*, pp. 290-305, Appleton and Lange, Connecticut.
- Gorska M, Lopaczyski W, Kinalska I. (1989) Effect of acute hyperthyroidism on insulin removal in rats. *Exp. Clin. Endocrinol.* 94, 345-350.
- Halliwell B, Gutteridge JMC. (1989) *Free radicals in Biology and Medicine*, pp. 188-276, Clarendon Press, Oxford.
- Hugget A, Nixon DA. (1957) Use of glucose oxidase, peroxidase and O-dianisidine in determination of blood and urinary glucose. *Lancet* 2, 368-370.
- Jamall IS, Smith C. (1985). Effects of cadmium on glutathione peroxidase, superoxide dismutase and Lipid peroxidation in isolated rat hepatocytes. *Toxicology Applied Pharmacology* 80, 33-42.
- Kar A, Panda S. (2003). Ayurvedic Therapies for Thyroid Dysfunction. In: *Scientific Basis of Ayurvedic Therapies*, edited by Mishra L, p. 194 CRC press, USA, Boca Raton, Florida.
- Kar A, Panda S, Bharti S. (2002) Relative efficacy of

- three medicinal plant extracts in the alteration of thyroid hormone concentrations in male mice. *J. Ethnopharmacol.* **81**, 281-285.
- Kreps EM, Tyurin VA, Gorbunov NV, Maximovich AA, Polyakov VN, Plyusnin VV, Kagan VE. (1986). Activation of lipid peroxidation on *Oncorhynchus gorbuscha* during spawning migration stress: a possible mechanism of adaptation. *Dokl. Akad. Nauk.* **286**, 1009-1012.
- Lowry OH, Rosebrough NJ, Farr AL., Randal RJ. (1951) Protein measurement with the Folin phenol reagent, *J. Biochem.* **193**, 265-275.
- Marklund S, Marklund G. (1974) Involvement of superoxide anion radical in the autoxidation of pyrogallol: a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47**, 469-474.
- Mirsalis JC, Hamilton CM, Schindler JE, Green CE, Dabbs JE. (1993) Effects of soya bean flakes and liquorice root extract on enzyme induction and toxicity in B6C3F1 mice. *Food Chem. Toxicol.* **31**, 343-350.
- Ohkawa H, Ohishi N, Yagi K. (1999) Assay of Lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358.
- Panda S, Kar A. (1998) *Ocimum sanctum* in the regulation of thyroid function in male mouse. *Pharmacol. Res.* **38**, 107-110.
- Panda S, Kar A. (1999) Guggulu (*Commiphora mukul*) induces triiodothyronine production: possible involvement of lipid peroxidation. *Life Sci.* **65**, 137-141.
- Panda S, Kar, A. (2000) Regulation of hyperthyroidism by *R. serpentina* root extract in mice. *Phram. Pharmacol. Commun.* **6**, 517-520.
- Panda S, Kar A. (2001) Inhibition of T₃ production in Levo-thyroxine-treated female mice by the root extract of *Convolvulus pluricaulis*. *Hormone and Metabolic Research* **33**, 16-18.
- Panda S, Kar A. (2003) Possible amelioration of hyperthyroidism by the leaf extract of *Annona squamosa*. *Curr. Sci.* **84**, 1402-1403.
- Panda S, Bharti S, Kar A. (2002) *Emblica officinalis* and *Bauhinia purpurea* in the regulation of thyroid function and lipid peroxidation in male mice. *J. Herbs Spices Med.* **10**, 1-9.
- Pari L, Saravanan G. (2002) Antidiabetic effect of Cogent db, a herbal drug in alloxan- induced diabetes mellitus. *Comp. Biochem. Physiol.* **131**, 19-25.
- Rao VV, Dwivedi SK, Swarup D, Sharma SR. (1995). Hypoglycemic & antihyperglycaemic effects of *A. marmelos* leaves in rabbits. *Curr. Sci.* **69**, 932-933.
- Roith DL, Taylor SI, Olefsky JM. (1996) *Diabetes Mellitus: A fundamental and clinical text*, pp. 500-501, Lipponcot-Raven Publishers, Philadelphia.
- Seema PV, Sudha B, Pandayatti PS, Abraham A, Raghu KG, Paulose CS. (1996) Kinetic studies of purified malate dehydrogenase in liver of streptozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelos* (L) Correa ex Roxb. *Ind. J. Exp. Biol.* **34**, 600-602.
- Seiss H. (1993) Strategies of antioxidant defense. *Eur. J. Biochem.* **215**, 213-219.
- Sharma MK, Kumar M, Kumar A. (2002) *Ocimum sanctum* aqueous leaf extract provides protection against mercury-induced toxicity in Swiss albino mice. *Indian J. Exp. Biol.* **140**, 1079-1082.
- Tahiliani P, Kar A. (2003a) Mitigation of Thyroxine induced hyperglycaemia by two plant extracts. *Phytother. Res.* **13**, 11-15.
- Tahiliani P, Kar A. (2003b) Combined effects of *Trigonella* & *Allium* extracts in the regulation of hyperthyroidism. *Phytomedicine* **10**, 665-668.
- Visser TJ, Fekkes D, Docter R, Hennemann G. (1978) Sequential deiodination of thyroxine in rat liver homogenate. *Biochem. J.* **174**, 221-229.
- Williamson EM. (2001) Synergy and other interactions in phytomedicine. *Phytomedicine* **8**, 401-409.