

IN VITRO STUDIES ON THYROGENIC EFFECT OF COMMIPHORA MUKUL (GUGGULU)

A.K. SINGH, G.C PRASAD AND S.N. TRIPATHI

*Department of Shalakya, Institute of medical Sciences,
Banaras Hindu University, Varanasi – 221005 India*

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ABSTRACT : *The results of this study has revealed a significant increase in I^{131} uptake of cultivated thyroid gland in the media containing petroleum ether extract of Commiphora mukul. Besides, T_3 resin uptake, protein bound iodine as well as free thyroxine index in the media are also observed to be significantly ($P 0.001$) increased. When explants are cultivated in the media containing melatonin, there is significant depression in thyroid I^{131} uptake and media T_3 resin uptake, protein bound iodine as well as free thyroxine index. Further, the inhibitory action of melatonin has been antagonized when similar explants are cultivated in the media containing melatonin and P.E. extract of c. mukul together thus, C. mukul may be useful in the management of hypothyroidism and its associated disorders like, hypercholesterolemia, hyperlipidemia, atherosclerosis etc.*

Introduction

Lipoprotein metabolism is known to be affected by alterations in the level of thyroid functions in human as well as in rats where hypercholesterolemia has been consistently demonstrated in hypothyroidism (Story et. al. 1974, Byers et. al. 1973, Mathe et. al. 1976 and Kutly et. al.1978). Commiphora mukul has been proved to be a potent hypolipidemic, hypocholesterolaemic and antiatherosclerotic agent in clinical as well as experimental studies (Tripathi et. al. 1968, Satyavati et. al. 1969, Nityanand et. al. 1974 & 1975). Different fractions of this drug were used in the prevention of experimentally induced hypercholesterolemia in chick and maximum activity was recorded in the

petroleum ether extract and effective component of this fraction was identified as a ketosteroid with the molecular formula $C_{21}H_{30}O_3$ (Tripathi 1973). However, the hypocholesterolaemic and hypolipidemic action of this drug has been reported through thyroid gland from our in vivo studies. Nevertheless, the mode of action of this drug on thyroid has not yet been clearly understood. It has been proposed that its stimulatory effect on thyroid might have been exerted via pituitary or hypothalamus or it may have a direct action, Thus, it was intended to investigate the action of C. mukul on thyroid gland in tissue culture. Besides, the thyroid stimulating capacity of this drug has also been found out using an antithyroid agent.

Material and Methods

Young male mice (about 6-7 weeks old) were employed in this study and their thyroid glands were carefully dissected out under aseptic condition, washed thoroughly with Tyrode Solution after removing its extra-adherent tissue and then grown in vitro (Fell & Weiss 1965) in modified dulbecco's eagles medium (BIOS) supplement with 10% human serum (heat inactivated at 56°C) at 37.5°C in a water jacket incubator and under the gas phase of 50% oxygen, 45% nitrogen and 5% Co₂. Further, thyroid gland was also cultivated in the media containing different doses of melatonin (0.25 ng/ml and 0.50 ng/ml) and P.E. extract of *C. mukul* (100 ng/ml and 200 ng/ml). The most effective dose of these chemicals were selected and a separate media was prepared where the effective dose of melatonin (0.50 ng/ml) and P.E. extract of *C. mukul* (200 ng/ml) were added together.

Thyroid gland was cultivated for eight days and the I¹³¹ uptake of cultured explants was studied after introducing 1.0μci I¹³¹ into the medium 4 hours prior to harvesting them. The percentage I¹³¹ uptake was calculated per minute. The used culture media was collected on each alternate days and subjected for the estimation of T₃ resin uptake (Clark 1963) and protein bound iodine (Acland 1957) which were based on our observations that released

iodothyronines bind with media proteins under in vitro conditions. Further, media free thyroxine index was calculated (Clark 1965) which corresponds to the actual free thyroxine level.

Results

I¹³¹ uptake of the cultivated explants was observed to be maximum i.e. 3.65±0.28 on the fourth day in the normal culture. However, there was a decline in I¹³¹ uptake when the explants were grown in the media containing melatonin. In the case of low dose of melatonin (0.25 ng/ml) I¹³¹ uptake was minimum i.e. 2.25±0.19 on the 8th day after culture and was significant (P<0.02) whereas in the high dose, I¹³¹ uptake was maximally depressed i.e. 1.65 ± 0.28 on the 2nd day and was significant (P<0.01) as compared to control. Further there was a significant increase in I¹³¹ uptake when the explants were cultivated in the media containing P.E. extract of *C. mukul*. The increase in I¹³¹ uptake was dose related i.e. it was much more pronounced and significant (P< 0.001) in the case of high dose (200 μg/ml) of P.E. extract of *C. mukul*. A significant increase in I¹³¹ uptake of culture explants was also observed when thyroid gland of similar animals were grown in the media containing both melatonin as well as P.E. extract of *C. mukul* (Table 1).

TABLE I**I¹³¹ Uptake of Thyroid Gland After Different Treatment in Vitro (% Uptake; Mean ± SD)**

| Groups | Days after Culture | | | |
|---|--------------------|---------------|----------------|---------------|
| | II | III | IV | VIII |
| Control | 2.5 ±0.17 | 3.95 ±0.28 | 3.35 ±0.36 | 2.85 ±0.12 |
| Melatonin 0.25ng/ml | 2.41 ±0.27 | 3.5 ±0.37 | 2.75* ±0.30 | 2.25** ±0.19 |
| 0.50 ng/ml | 1.65***± 0.28 | 2.88* ±0.58 | 3.18 ±0.29 | 2.65 ±0.31 |
| P.E extract of C. mukul 100 µg/ml | 3.19 ±0.21 | 4.45±0.26 | 4.74±0.05 | 3.98±0.41 |
| 200 µg/ml | 5.15****±0.03 | 6.15****±6.61 | 6.15****±0.32 | 5.45****±0.17 |
| P.E extract of C. mukul 200 µg/ml + Melatonin 0.05 ng/ml | 3.31**±0.11 | 5.25***±0.22 | 5.75 ****±0.31 | 4.55****±0.23 |

- *P < 0.05 as compared to control value
** P < 0.02 as compared to control value
*** P < 0.01 as compared to control value
**** P < 0.001 as compared to control value

Maximum T₃ resin uptake in the used culture media of normal culture was observed on the 4th day after culture and it was 43.1 ± 1.7, but in the melatonin treated group of explants there was a gradual decline in T₃ resin uptake upto 6th day after culture and it followed a little increase on the 8th day. However, the depression in T₃ resin uptake was more significant in the case of high dose of melatonin (0.50 ng/ml). when thyroid gland was cultivated in the media containing P.E. extract of C. mukul, there was a gradual increase in media T₃ resin uptake which was more elevated and highly significant (P. 0.001) in the high dose. An increasing T₃ resin uptake was further observed when the explants were cultivated in the media containing melatonin as well as P.E. extract of C. mukul (Table 2).

The media protein bound iodine (PBI) of the unused culture media was 1.8 ± 0.04, However, in the control group the media PBI was almost double of the value obtained for unused culture media. In the melatonin treated group, the media PBI was observed to decline. Maximum depression was found on the 4th day in the high dose of melatonin and it did not change much in the later period. In the case of P.E. extract of C. mukul treated group of explants the media PBI increased gradually and more pronounced and significant increase was observed in the high dose (Table 3).

The calculated free thyroxine index of the media in the melatonin treated group was observed to undergo a decline which was highly significant (P< 0.001) in the high dose as compared to control. However, media free thyroxine index was observed to

increase in the C. Mukul treated group and the increase was pronounced and significant in the high dose (200 $\mu\text{g/ml}$). further, increased free thyroxine index was also observed in the group where explants were cultivated in the media containing melatonin as well as P. E. extract of C. mukul together (Table 4).

Discussion

This study has delineated that melatonin treatment to culture explants brought about a decline in I^{131} uptake of the cultivated thyroid gland where it is significant only in the early period of culture in the case of high dose while in the later period in the case of low dose or melatonin. However, such variation could be due to dose response of melatonin. Besides, there is highly significant depression in T_3 resin uptake, PBI and free thyroxine index in the media. It indicates that melatonin affects hormone releasing activity of thyroid gland more significantly than that of iodide accumulating capacity. However, our results confirm that the inhibitory action of melatonin on thyroid function is directly exerted at the cell level. Regarding the mechanism through which melatonin influences thyroid activity is not yet clear. However, our earlier findings shows that

melatonin suppresses thyroid function through specific melatonin binding proteins in thyroid gland (Singh et. al. 1981).

On the other hand the explants cultivated in the media containing P.E. extract of C. mukul has revealed a significant increase in I^{131} uptake of cultivated thyroid explants as well as T_3 resin uptake, PBI and free thyroxine index in the media. These results of this study suggest that C. mukul stimulates thyroid function which is directly exerted at the cell level. Further, the inhibitory effect of melatonin has been completely antagonized when the similar explants have been grown in the media containing melatonin as well as P.E. extract of C. mukul. Thus, it again confirms that C. mukul is capable to stimulate the thyroid cell directly and the stimulating capacity of this drug is so much intense that it fully counteracts the inhibitory action of melatonin. Since biosynthesis of thyroid hormones is mediated through many enzymes, so it is most likely that the stimulatory effect of C. mukul might involve one or the other enzyme (S) responsible for the thyroid hormone biogenesis. Thus, this drug may prove to have a good scope in the management of hypothyroidism and its associated disorders.

TABLE II**T3- Resin Uptake of Media After Different Treatment to Thyroid Gland in Vitro****(% Uptake; Mean ± SD)**

| Groups | Days after Culture | | | |
|---|--------------------|--------------|----------------|---------------|
| | II | III | IV | VIII |
| Control | 38.0 ±3.4 | 43.1 ±1.7 | 41.5 ±0.5 | 38.7 ±1.7 |
| Melatonin 0.25ng/ml | 37.1 ±3.9 | 35.1* ±4.3 | 33.5** ±2.3 | 36.3 ±2.7 |
| 0.50 ng/ml | 31.2*± 1.7 | 30.5*** ±3.6 | 29.7***** ±2.7 | 34.5** ±1.1 |
| P.E extract of C. mukul 100 µg/ml | 39.0 ±1.8 | 44.1±2.0 | 47.5*****±0.05 | 3.98±0.41 |
| 200 µg/ml | 47.3±2.9 | 54.0±3.0 | 56.6*****±2.1 | 52.2*****±1.4 |
| P.E extract of C. mukul 200 µg/ml + Melatonin 0.05 ng/ml | 36.59±2.15 | 45.13±3.12 | 48.59**±2.56 | 46.43***±3.17 |

*P < 0.05 as compared to control value
 ** P < 0.02 as compared to control value
 *** P < 0.01 as compared to control value
 **** P < 0.001 as compared to control value

TABLE III**Media PBI After Different Treatment to Thyroid Gland in Vitro****(µg % Uptake; Mean ± SD)**

| Groups | Days after Culture | | | |
|---|--------------------|-------------|----------------|---------------|
| | II | IV | VI | VIII |
| Control | 4.10 ±0.71 | 4.42 ±0.97 | 4.81 ±0.26 | 4.62 ±0.22 |
| Melatonin 0.25ng/ml | 40.5 ±0.21 | 3.80 ±0.93 | 3.41 ±0.86 | 2.92 ±10.31 |
| 0.50 ng/ml | 3.15± 0.26 | 2.71 ±0.25 | 2.93*** ±0.83 | 2.72*** ±0.57 |
| P.E extract of C. mukul 100 µg/ml | 5.15 ±0.57 | 5.18±0.20 | 6.21***±0.19 | 6.28***±0.20 |
| 200 µg/ml | 6.23*±0.19 | 6.36**±0.26 | 7.63*****±0.21 | 7.09±0.30 |
| P.E extract of C. mukul 200 µg/ml + Melatonin 0.05 ng/ml | 3.28±0.19 | 5.31±0.21 | 5.78±0.32 | 4.49±0.31 |

*P < 0.05 as compared to control
 ** P < 0.02 as compared to control
 *** P < 0.01 as compared to control
 **** P < 0.001 as compared to control

TABLE IV

**Free Thyroxine Index of Media After Different Treatment to Thyroid Gland in Vitro
 (Mean ± SD)**

| Groups | Days after Culture | | | |
|---|--------------------|----------------|----------------|----------------|
| | II | IV | VI | VIII |
| Control | 5.22 ±0.28 | 6.53 ±0.68 | 6.80 ±0.47 | 6.07 ±0.88 |
| Melatonin 0.25ng/ml | 4.97 ±0.27 | 4.50** ±0.47 | 3.91**** ±0.32 | 3.53**** ±0.19 |
| 0.50 ng/ml | 3.25****± 0.33 | 2.83**** ±0.23 | 2.60**** ±0.61 | 3.09**** ±0.18 |
| P.E extract of C. mukul 100 µg/ml | 6.01±0.35 | 7.96±0.61 | 5.96**±0.27 | 9.19***±0.36 |
| 200 µg/ml | 9.28**±0.25 | 11.53***±1.2 | 14.07****±0.67 | 12.76****±0.63 |
| P.E extract of C. mukul 200 µg/ml + Melatonin 0.05 ng/ml | 6.54±0.79 | 9.33**±0.89 | 11.35****±1.09 | 10.98****±0.77 |

*P < 0.05 as compared to control value
 ** P < 0.02 as compared to control value
 *** P < 0.01 as compared to control value
 **** P < 0.001 as compared to control value

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