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CLINICAL STUDY OF MARKETED AYURVEDIC PREPARATION IN THYROID INDUCED STRESS

A. A. ROY*[1], K. R. KAKADE[2], R.P. BHOLE[1], V. B. MATHUR[1]

[1]Sharad Pawar College of Pharmacy, Wanadongri, Hingana road, Nagpur-441110 (M. S.), INDIA.

[2]Dadasaheb Balpande College of Pharmacy, Near Swami Samarth Dham Mandhir, Vesa, Hudhkheswer, Nagpur- 440034 (M.S.), INDIA.

E-mail address: ritesh.edu@gmail.com

Tel.: +91-7104-236352,Fax: +91-7104-235084

ABSTRACT : Vitalex, a marketed Ayurvedic preparation is being used extensively for the treatment of stress and strain syndrome, metabolic disorders, hormonal disturbance, chronic illness, etc. Literature survey revealed involvement of oxidative stress in several diseases. Oxidative stress can be reduced by using antioxidant. Therefore, it was decided to carry out clinical study of this marketed Ayurvedic preparation to assess its antioxidant property and its use in thyroid disorders. Clinical study was carried out in healthy volunteers as well as in diseased patients by considering two parameters namely behavioural and oxidative stress parameters. Behavioural parameters included anxiety, depression, acidity, constipation etc. and these parameters were further marked as mild, moderate and severe according to the intensity. Oxidative stress parameters included lipid peroxidation (LPO), reduced glutathione content (GSH), superoxide dismutase (SOD) and catalase activity (CAT). The protocol was approved by the Institutional Ethical Committee at J. N. Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha. Both parameters were studied before and after treatment with the marketed Ayurvedic preparation. After completion of protocol of behavioural parameters, blood was withdrawn from hypothyroid and hyperthyroid patients and was analyzed for various oxidative stress parameters. The present finding revealed decrease in LPO level and increase in level of GSH, SOD and CAT activity by the treatment of Vitalex, indicating reduction in oxidative stress in hypothyroid and hyperthyroid state. Thus, it was concluded that the Vitalex had antioxidant property because of which there was reduction in oxidative stress.

KEY WORDS: Clinical study, Oxidative stress, antioxidant property, marketed Ayurvedic preparation, Diseased-induced stress

1. INRODUCTION

Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules which are generally very reactive.[1] Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue or organ, caused by reactive oxygen species (ROS).[2] Oxidative stress is the cause of many human diseases like diabetes, thyroid disorders, hypertension, arthritis etc.[3] Antioxidant supplementation are proven to reduce oxidative stress to a greater extent.[4] Antioxidants are compounds which act as inhibitors of the oxidative damage. They are quite large in number and diverse in nature, which oppose the process of oxidation largely by neutralizing free radicals and at relatively small concentration have the potential to inhibit the oxidants chain reaction.[5] Vitalex, a marketed Ayurvedic preparation is being used extensively for the treatment of stress and strain syndrome, metabolic disorders, hormonal disturbance, chronic illness etc. Therefore, it was decided to carry out clinical study of this marketed Ayurvedic preparation to assess its antioxidant property and its use in thyroid induced stress.

2. MATERIALS AND METHODS

The work was carried out at Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha. The Institutional Ethical Committee in its meeting had approved the research work proposed to be carried out at J. N. Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha. This work was carried out in accordance with ethical guidelines

prescribed by Central Ethics Committee on Human Research (C.E.C.H.R). Vitalex was procured as gift sample from Vaidik Remedies, Wadi, Nagpur. All the chemicals which were used for this study were of analytical grade. The patient history was noted with the help of physician, to correlate stress and diabetes. The behavioral parameters related were anxiety, depression, acidity, constipation, pain, body temperature and blood pressure. Blood sample from hypothyroid and hyperthyroid patients as well as from healthy volunteers were obtained before and after the dosage regimen of Vitalex. Evaluation of oxidative stress parameters were done by various conventional methods such as lipid peroxidation (LPO) [6], reduced glutathione content (GSH) [7], superoxide dismutase (SOD) [8] and catalase activity (CAT) [9].

2.1. Lipid peroxidation assay:

For determination of lipid peroxidation (LPO), the blood was withdrawn from retro-orbital plexus and was taken in the centrifuge tube containing anticoagulant. From this 5% suspension of RBC in 0.1 M phosphate buffered saline was prepared. To the 2 ml of this 5% suspension, 2 ml of 28% trichloroacetic acid was added and centrifuged. After centrifugation the supernatant was separated. To the 4 ml of supernatant 1 ml of 1% thiobarbituric acid was added, heated in boiling water for 60 minutes and cooled immediately. The absorbance was measured spectrophotometrically at 532 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) (1.56×10^5) and expressed in terms of nanomoles of MDA/g Hb.

2.2. Reduced glutathione assay:

Glutathione activity was measured in whole blood. The blood (0.2 ml) was added to 1.8 ml of distilled water followed by 3.0 ml of precipitating mixture (1.67gms of metaphorsphoric acid, 0.2gms of EDTA, 30gms NaCl to make 100 ml of solution). It was centrifuged at 2000 rpm for 5 minutes. Supernatant (1 ml) was added to 1.5 ml of phosphate solution followed by addition of 0.5 ml of DTNB reagent. The optical density was measured at 412 nm using spectrophotometer.

2.3. Superoxide dismutase assay:

The activity of SOD was determined in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 µl of the lysate, 2 ml of 75 mM of Tris-HCl buffer (pH 8.2), 0.6 ml of 30 mM of EDTA and 0.3 ml of 2 mM of pyrogallol were added. An increase in the absorbance was measured at 420 nm for 3 minutes using spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of auto-oxidation of pyrogallol, as determined by change in absorbance/minute at 420nm.

2.4. Catalase assay:

The activity of catalase enzyme was determined in erythrocyte lysate. The lysate (50 µl) was taken and added to a test tube containing 2 ml of phosphate buffer (pH 7.0) and then 1 ml of 30 mM of H_2O_2 was added to it. The decrease in absorbance was measured at 240 nm for 1 minute using spectrophotometer.

3. RESULTS

3.1. Statistical analysis:

Values were expressed as mean±SEM (n=6). All data were analysed by One-Way ANOVA followed by Newman-Keuls multiple comparison test. The level of significance was considered at $p<0.05$ and $p<0.01$. The results of study were divided into two parameters:

3.1.1. Behavioural parameters

3.1.2. Oxidative stress parameters

3.1.1. Behavioural parameters

These parameters were studied before and after the dosage regimen of Vitalex and the results are shown in Table.1, 2, 3, 4, 5 and 6. According to behavioural study, the normal healthy individuals were almost free from anxiety, depression, acidity, constipation etc., whereas hypothyroid and hyperthyroid patients exhibited these parameters. According to oxidative stress parameters, there were no significant changes with normal volunteers, whereas hypothyroid and hyperthyroid patients showed significant reversal after the treatment with Vitalex.

3.1.2. Oxidative stress parameter:

3.1.2.1. Lipid peroxidation assay:

In normal control group the LPO levels (0.8342 ± 0.1381) showed insignificant decrease in Vitalex treated control group; whereas in hypothyroid and hyperthyroid group, the LPO levels (6.703 ± 0.9836) and (10.15 ± 0.9085) respectively showed significant decrease with the treatment of Vitalex. The results are shown in Table 7. Thus, the marked increase in the oxidative stress was found in hypothyroid and hyperthyroid groups as indicated by increase in LPO levels, whereas treatment with Vitalex showed reduction in oxidative stress.

3.1.2.2. Reduced glutathione assay:

In normal control group the GSH content (2.079 ± 0.3177) showed insignificant increase in Vitalex treated control group; whereas hypothyroid and hyperthyroid group, the GSH content (0.1468 ± 0.0233) and (0.1096 ± 0.0143) respectively showed significant increase with the treatment of Vitalex. The results are shown in Table 8. Thus, the marked increase in the oxidative stress was found in hypothyroid and hyperthyroid groups as indicated by decrease in GSH content, whereas treatment with Vitalex showed decrease in oxidative stress as indicated by the increased GSH content.

3.1.2.3. Superoxide dismutase assay:

In normal control group the SOD activity (5.375 ± 0.8135) showed insignificant increase in Vitalex treated control group; whereas in hypothyroid and hyperthyroid group, the SOD activity (0.4836 ± 0.1236) and (0.4783 ± 0.1060) showed significant increase with the treatment of Vitalex. The results are shown in Table 9. Thus, the marked increase in the oxidative stress was found in hypothyroid and hyperthyroid groups as indicated by

decreased in SOD activity, whereas treatment with Vitalex showed decrease in oxidative stress as indicated by the increased SOD activity as compared to hypothyroid and hyperthyroid groups.

3.1.2.4. Catalase assay:

In normal control group the CAT activity (2.935 ± 0.7430) showed insignificant increase in Vitalex treated control group; whereas in hypothyroid and hyperthyroid groups, the CAT activity (0.3717 ± 0.1226) and (0.2288 ± 0.09189) showed significant increase with the treatment of Vitalex. The results are shown in Table 10. Thus, the marked increase in the oxidative stress was found in hypothyroid and hyperthyroid groups as indicated by decrease in CAT activity, whereas treatment with Vitalex showed decrease in oxidative stress as indicated by the increased CAT activity as compared to hypothyroid and hyperthyroid groups.

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4. DISCUSSION

The major objective of the work was to assess the free radical scavenging activity of the selected marketed Ayurvedic preparation (Vitalex) by evaluation of free radical scavenging activity and oxidative stress parameters. The results of behavioural parameters suggested lowering of the stressful condition in vitalex treated volunteers. The results of lipid peroxidation and antioxidant enzyme assay indicated that there was marked increase in the level of lipid peroxidation and decrease in the level of antioxidant enzyme in hypothyroid and hyperthyroid groups, whereas the level of malondialdehyde (MDA) was lowered and the levels of antioxidant enzymes were increased in both diseased conditions when treated with Vitalex. This suggested that the Vitalex had marked free radical scavenging activity and because of which, Vitalex showed utility in amelioration of diseased induced stress.

Thus, it can be speculated that the free radical scavenging activity of Vitalex, may be attributed to the increase in the level of endogenous antioxidant enzymes system.

Our studies indicated that the vitalex had antioxidant property because of which it reduced the oxidative stress generated in diseased conditions. Therefore this drug is used for the treatment of stress and strain syndrome, metabolic disorders, hormonal disturbances, chronic illness etc. More biochemical studies are required to understand the potential benefits of antioxidants preparation in the prevention and/or treatment of diseases.

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Table 1. Normal volunteers (Studied before treatment with Vitalex)

Sr. No.	Anxiety	Depression	Acidity	Constipation	Pain	Body-temp (^{0}F)	Blood pressure (mmHg)
1	--	--	--	+	+	98.2	80/125
2	--	--	+	--	+	98.6	95/135
3	--	--	--	--	--	97.6	90/128
4	--	--	--	--	--	97.2	70/125
5	--	--	--	--	--	97.4	90/120
6	--	--	--	+	+	98.2	75/110

Table 2: Normal volunteers (Studied after treatment with Vitalex)

Sr. No.	Anxiety	Depression	Acidity	Constipation	Pain	Body-temp (^{0}F)	Blood pressure (mmHg)
1	++	+	++	+++	++	98.4	70/100
2	+	--	--	--	--	98.4	90/125
3	+	+	++	+	+	98.6	70/110
4	++	--	+	+	+	98.2	85/120
5	++	--	++	--	+	98.2	70/100
6	--	--	++	++	++	99.2	85/130

(+) Mild, (++) Moderate, (+++) Severe

Table 3. Hypothyroid volunteers (Studied before treatment with Vitalex)

Sr. No.	Anxiety	Depression	Acidity	Constipation	Pain	Body-temp (^{0}F)	Blood pressure (mmHg)
1	++	+	++	+++	++	98.4	70/100
2	+	--	--	--	--	98.4	90/125
3	+	+	++	+	+	98.6	70/110
4	++	--	+	+	+	98.2	85/120
5	++	--	++	--	+	98.2	70/100
6	--	--	++	++	++	99.2	85/130

Table 4. Hypothyroid volunteers (Studied after treatment with Vitalex)

Sr. No.	Anxiety	Depression	Acidity	Constipation	Pain	Body-temp (^{0}F)	Blood pressure (mmHg)
1	+	+	+	++	+	97.4	70/120
2	--	--	+	--	--	98.2	85/125
3	--	--	+	--	--	97.2	90/125
4	+	--	+	--	--	97.2	85/120
5	+	--	+	--	--	97.4	90/120
6	--	--	+	+	+	98.2	90/125

(+) Mild, (++) Moderate, (+++) Severe

Table 5. Hyperthyroid volunteers (Studied before treatment with Vitalex)

Sr. No.	Anxiety	Depression	Acidity	Diarrhoea	Pain	Body-temp (^{0}F)	Blood pressure (mmHg)
1	++	+	++	+++	++	99.2	100/130
2	+	--	--	--	+	98.8	100/130
3	+	+	++	+	+	99.2	95/130
4	++	--	+	+	++	99.2	100/135
5	++	--	++	--	+	99.2	102/140
6	--	--	++	++	+	100.2	100/140

Table 6. Hyperthyroid volunteers (Studied before treatment with Vitalex)

Sr. No.	Anxiety	Depression	Acidity	Diarrhoea	Pain	Body-temp (^{0}F)	Blood pressure (mmHg)
1	++	+	--	+	+	98.2	90/125
2	--	--	+	+	+	98.2	90/125
3	+	--	+	--	--	98.4	90/125
4	--	+	--	+	+	98.4	90/125
5	+	--	--	++	+	98.6	90/130
6	+	--	--	+	+	99.2	90/120

(+) Mild, (++) Moderate, (+++) Severe

Table 7. Lipid peroxidation level

Groups	**Lipid peroxidation (nM of MDA/gHb)**	
	Normal	**Vitalex treated**
Control	0.8342 ± 0.1381	0.8299 ± 0.1084
Hypothyroid	6.703 ± 0.9836*	2.880 ± 0.4695**
Hyperthyroid	10.15 ± 0.9085*	3.392 ± 0.5833**

Values are mean ± SEM, (n=6). *$p<0.05$, when compared to respective normal group.
**$p<0.01$, when compared to respective diseased group (hypothyroid and hyperthyroid).

Table 8. Reduced glutathione level

Group	Reduced glutathione (µm DTNB conjugated/gHb)	
	Normal	Vitalex treated
Control	2.079 ± 0.3177	2.091 ± 0.2469
Hypothyroid	0.1468 ± 0.0233*	0.7780 ± 0.1150**
Hyperthyroid	0.1096 ± 0.0143*	0.7632 ± 0.1251**

Values are mean ± SEM, (n=6). *p<0.05, when compared to respective normal group.
**p<0.01, when compared to respective diseased group (hypothyroid and hyperthyroid).

Table 9. Superoxide dismutase activity

Group	Superoxide dismutase (units/mg protein)	
	Normal	Vitalex treated
Control	5.375 ± 0.8135	5.450 ± 0.9238
Hypothyroid	0.4836 ± 0.1236*	2.762 ± 0.3176**
Hyperthyroid	0.4783 ± 0.1060*	2.732 ± 0.3273**

Values are mean ± SEM, (n=6). *p<0.05, when compared to respective normal group.
**p<0.01, when compared to respective diseased group (hypothyroid and hyperthyroid).

Table 10. Catalase assay

Group	Catalase (units/mg protein)	
	Normal	Drug treated
Control	2.935 ± 0.7430	2.998 ± 0.4531
Hypothyroid	0.3717 ± 0.1226*	1.822 ± 0.2215**
Hyperthyroid	0.2288 ± 0.09189*	1.689 ± 0.2824**

Values are mean ± SEM, (n=6). *p<0.05, when compared to respective normal group.
**p<0.01, when compared to respective diseased group (hypothyroid and hyperthyroid).

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