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RESEARCH ARTICLE

COMPARATIVE STUDY OF TRIPHALA AND TRIKATU ON OBESITY INDUCED OXIDATIVE STRESS IN HEART OF ALBINO RATS

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ABSTRACT

Objective: The purpose of the present study was to evaluate the cardioprotective and antioxidant properties of Triphala and Trikatu against obesity induced cadiotoxicity in female albino rats. This was compared with standard herbal (ayurslim) and synthetic (sibutrex10TM) anti-obesity drugs.

Methods: Animals were divided in to three groups. Group I: Control group, Group II: Short duration group, Group III: Long duration group. Group II and Group III were further sub-divided into six sub groups, each group consisting of five animals. 1. Obesity control - The animals received atherogenic diet as an oral dose. 2. Obesity + Triphala - The animals received atherogenic diet + 8.3 mg triphala / 100g body weight / day, as an oral dose. 3. Obesity + Trikatu - The animals received atherogenic diet + 4.16 mg. of trikatu /100g. body weight / day, as an oral dose. 4. Obesity + Triphala and Trikatu - The animals received atherogenic diet + 4.15 mg of triphala and 2.08 mg of trikatu respectively/ 100g. body weight / day, as an oral dose. 5. Obesity + Ayurslim - The animals received atherogenic diet + 13.3mg. of ayurslim / 100g. body weight / day, as an oral dose. 6. Obesity + Sibutrex10TM - The animals received atherogenic diet + 0.17mg. of sibutrex10TM / 100g. body weight / day, as an oral dose.

Result: The present study showed that obesity had caused an significant ($P<0.05$) decrease in the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione in heart. There was significant ($P<0.05$) increase in lipid peroxidation in heart tissues.

Conclusion: The oral administration of Triphla and trikatu were more effective in the restoration of obesity induced oxidative stress in the heart.

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INTRODUCTION

Obesity is a global nutritional concern. The increasing prevalence of overweight, obesity and its consequences promoted the World Health Organization (WHO) to designate obesity as a global epidemic (WHO, 1998). Oxidative stress has been repeatedly addressed as an important mechanism of cytotoxicity and indirect genotoxicity that may be considerably increased in certain pathologies (Kirkland and Muller, 2000; Pratt and Barron, 2003). It is triggered by exposure to exogenous factors or by chemicals producing reactive oxygen species and is associated with an over production of ROS, as well as impairment of anti-oxidant defensive capacity. Obesity has been shown to be one of the conditions that decrease anti-oxidant capacity (Carmiel Haggai *et al*., 2003). Patients with type 2 diabetes mellitus showed an increase in lipid peroxidation from the onset of disease (Sundaram *et al*., 1996). Previous reports indicate that lipid peroxidation is elevated in both liver and plasma in humans and obese animals (Desci *et al*., 1997). Furthermore, diets rich in fats (either saturated or unsaturated) are also associated with increased lipid peroxidation in several tissues, such as aorta, liver and plasma (Folch *et al.*, 1957; Koneru *et al*., 1995). Obesity is associated with lower plasma or tissue levels of anti-oxidants and /or increased susceptibility to injury during oxidative changes *in vitro* (Koneru *et al*., 1995; Vincent *et al*., 1998). Triphala, comprising three fruits, *Terminalia bellerica*, *Terminalia chebula* and *Emblica officinalis*, is one of the most common herbal formulations used in Indian Ayurvedic medicine. It is believed to promote health, render immunity

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and rejuvenate (Nadkarni, 1976). The antioxidant rich herbal formulation is used in the treatment of several conditions like jaundice, asthma, constipation, fever, fatigue, anemia, vomiting, typhoid, chronic ulcers, and eye diseases, as well as in the treatment of infectious diseases such as tuberculosis, pneumonia and acquired immune deficiency syndrome (Chouhan *et al*., 2013). Triphla and its components have diverse medicinal properties and have been shown to possess antibacterial, antifungal, anti malarial, antiviral, anticancer, anti-inflammatory, antioxidant, hepatoprotective and gastroprotective activity (Valsaraj *et al*., 1997). Trikatu is a traditional Ayurvedic herbal formulation consisting of three herbs in equal amounts, *Piper nigrum* (black pepper) *Piper longum* (Long pepper) and *Zingiber officinale* (ginger). 'Trikatu' is a Sanskrit word meaning 'three acrids' referring to the pungent qualities of the three ingredient herbs. The use in Ayurveda of these three herbs is documented in the ancient Ayurveda Materia medica dating back to several thousands of years (Johri and Zutshi, 1992). In Ayurveda, the ingredients of trikatu are important components of numerous formulations used for a wide range of disorders (Johri and Zutshi, 1992). The present study was undertaken to assess the protective effect of either Triphala or Trikatu or their combination in the obesity induced oxidative stress in heart. The standard weight reducing drugs (ayurslim and sibutrex) was used as a reference drugs for the purpose of comparison.

MATERIALS AND METHODS

Drugs and Chemicals

Sibutramine hydrochloride (sibutrex10^{TM}) was purchased from British Drug House, India. Triphala, trikatu and ayurslim from the Himalaya Drug Company, Makali, Bangalore, India.

Animals

Adult female albino rats of Wistar Strain weighing about 180 – 200g were procured from Kerala Agricultural University, Kerala and acclimatized to our animal house conditions for 2 weeks. The animals were housed in well-ventilated animal house with constant 12 $\pm$1 hours light and dark schedule. They were provided with standard diet and clean water *ad libitum*. Guidelines recommended by the committee for the Purpose of Supervision and Control of Experiments on Animals (CPSCEA), Government of India, were followed for the care and maintenance of the animals. The experimental procedure was approved Institutional Animal Ethical Committee (IAEC) (No. 722/02/a/CPCSEA).

Experimental Design

Atherogenic diet induced Hyperlipidemia in experimental rats:

Preparation of diet

Hyper caloric diet of Atherogenic diet was prepared by following constituents. The percentage is for 100g diet. The feed was prepared, dried and administered every day in morning to animals with water ad libitum. The Atherogenic diet was given for 30 days along with standard rat chow diet.
Atherogenic diet formula

1. Cholesterol – 2%
2. Cholic acid – 1%
3. Coconut oil – 2%

The animals were divided into three groups. Group I: Control group, received normal standard diet orally. Group II : Short duration group, rats were fed individually with triphala, ayurslim and sibutrex10^{TM} at12 hours intervals for thirty consecutive days. Group III : Long duration group, rats were fed individually with triphala, ayurslim and sibutrex10^{TM} at12 hours intervals for sixty consecutive days. Group II and Group III were further sub-divided into six sub groups, each group consisting of five animals. The animals received the following regimen of treatment, all the treatments were constructed on the basis of adult dosage prescribed by the physicians to humans and extrapolated to animal body weight. Group 1. Obesity control, received atherogenic diet /day as an oral dose. Group 2. Obesity + Triphala, received atherogenic diet + 4.15 mg. of triphala / Kg. body weight / day, as an oral dose. Group 3. Obesity + Trikatu, received atherogenic diet + 4.16 mg. of trikatu /100g. body weight / day, as an oral dose. Group 4. Obesity + Triphala and Trikatu, received atherogenic diet + 4.15 mg of triphala and 2.08 mg of trikatu respectively/ 100g. body weight / day, as an oral dose. Group 5. Obesity + Ayurslim, received atherogenic diet + 13.3mg. of ayurslim / Kg. body weight / day, as an oral dose. Group 6. Obesity + Sibutrex10^{TM}, received atherogenic diet + 0.17mg. of sibutrex10^{TM} / Kg. body weight / day, as an oral dose.

Animal sacrifice

The animals were weighed before and after treatment. Twenty four hours after the last treatment schedule the animals were sacrificed by decapitation method. The animals were dissected out and heart was removed. The organs were cleaned off adhering connective tissues and blood stains, washed in cold physiological saline thrice, blotted on a filter paper and weighed using electronic balance, wrapped in aluminum foil and stored at –20^{o}C in air tight glass containers until assayed for biochemical parameters.

Biochemical Parameters

The levels of lipid peroxidation (Nichens and Samuelson, 1968), oxidative enzymes like catalase (Sinha, 1972), superoxide dismutase (Kakkar *et al.*, 1984) and non - enzymatic anti-oxidant glutathione (Beutler and Kelly, 1963) were estimated in the heart tissue.

Statistical Analysis

The data of every experiments were statistically expressed as mean ± standard error of mean (SEM). The SEM was calculated by using the following formula (Kennedy and Neville, 1986). All the means observed by every treatment were compared by ANOVA (Alder and Roessler, 1977) and ranked by using Duncan's Multiple Range Test (DMRT) (Duncan, 1955) for analyzing the significance of treatments at 1% and 5% level.

Table 1. Effect of triphala, trikatu and their combination and standard anti-obesity drugs on Lipid peroxidation and Superoxide dismutase in heart of albino rats

Treatments	Lipid peroxidation (nmoles/min/mg protein)	Superoxide dismutase (nmoles / min /mg protein)
Group I – CONTROL	3.520 ± 0.03^{j}	8.360 ± 0.02^{c}
Group II – SHORT DURATION		
Obesity	$7.420 \pm 0.03^{**b}$	$4.860 \pm 0.02^{**i}$
Obesity + Triphala	$5.100 \pm 0.05^{**e}$	$6.220 \pm 0.03^{**f}$
Obesity + Trikatu	$6.140 \pm 0.02^{**c}$	$5.820 \pm 0.03^{**h}$
Obesity + Triphala & Trikatu	$4.880 \pm 0.03^{**fg}$	$7.380 \pm 0.03^{**d}$
Obesity + Ayurslim	$4.500 \pm 0.04^{**h}$	$8.280 \pm 0.33^{**c}$
Obesity + Sibutrex10™	$3.980 \pm 0.07^{**I}$	$4.840 \pm 0.02^{**j}$
Group III - LONG DURATION		
Obesity	$9.480 \pm 0.03^{**a}$	$3.380 \pm 0.03^{**k}$
Obesity + Triphala	$6.100 \pm 0.05^{**c}$	$8.320 \pm 0.03^{**c}$
Obesity + Trikatu	$7.340 \pm 0.02^{**b}$	$7.620 \pm 0.07^{**e}$
Obesity + Triphala & Trikatu	$5.820 \pm 0.03^{**d}$	$9.560 \pm 0.03^{**a}$
Obesity + Ayurslim	$5.000 \pm 0.04^{**ef}$	$8.860 \pm 0.33^{**b}$
Obesity + Sibutrex10™	$4.860 \pm 0.02^{**g}$	$6.060 \pm 0.07^{**g}$

Each value is the Mean ± SE of five animals
** Control Vs Treatment, significant at 1% level by ANOVA
Means ± SE followed by a common letter are not significantly different at the 5% level by DMRT (a, b, c etc).

Table 2. Effect of triphala, trikatu and their combination and standard anti-obesity drugs on Catalase and Glutathione in heart of albino rats

Treatments	Catalase (nmoles/min/mg protein)	Glutathione (nmoles/min/mg protein)
Group I – CONTROL	4.602 ± 0.02^{bc}	4.844 ± 0.004^{b}
Group II – SHORT DURATION		
Obesity	$3.604 \pm 0.02^{**g}$	$3.458 \pm 0.008^{**I}$
Obesity + Triphala	$3.868 \pm 0.03^{**f}$	$4.632 \pm 0.02^{**d}$
Obesity + Trikatu	$3.904 \pm 0.02^{**f}$	$4.468 \pm 0.009^{**f}$
Obesity + Triphala & Trikatu	$4.444 \pm 0.009^{**de}$	$4.820 \pm 0.01^{**b}$
Obesity + Ayurslim	$4.400 \pm 0.03^{**e}$	$4.880 \pm 0.004^{**a}$
Obesity + Sibutrex10™	$4.656 \pm 0.01^{**ab}$	$4.562 \pm 0.01^{**e}$
Group III - LONG DURATION		
Obesity	$3.048 \pm 0.05^{**h}$	$2.776 \pm 0.008^{**j}$
Obesity + Triphala	$3.684 \pm 0.02^{**g}$	$4.386 \pm 0.009^{**g}$
Obesity + Trikatu	$3.926 \pm 0.02^{**f}$	$4.178 \pm 0.007^{**h}$
Obesity + Triphala & Trikatu	$4.478 \pm 0.02^{**de}$	$4.548 \pm 0.01^{**e}$
Obesity + Ayurslim	$4.710 \pm 0.03^{**a}$	$4.762 \pm 0.01^{**c}$
Obesity + Sibutrex10™	$4.536 \pm 0.006^{**cd}$	$4.630 \pm 0.006^{**d}$

Each value is the Mean ± SE of five animals
** Control Vs Treatment, significant at 1% level by ANOVA
Means ± SE followed by a common letter are not significantly different at the 5% level by DMRT (a, b, c etc).

RESULTS

Obesity induced rats (Group 2) showed significant ($P<0.05$) increase in lipid peroxidation (Table-1) and significant reduction in the levels of the enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD) and non enzymatic antioxidants such as glutathione (Table-2). Triphala and trikatu combinations had caused a significant ($P<0.05$) reduction in the lipid peroxidation when compared to obese animals. The combination of triphala and trikatu significantly ($P<0.05$) restored the CAT, SOD and glutathione activity in short duration and in long duration groups like that of standard drugs.

DISCUSSION

Obesity increases the mechanical and metabolic load on the myocardium, thus increasing myocardial oxygen consumption. It is likely that, obesity induced increased myocardial oxygen consumption directly contributes to elevated lipid peroxidation (Girotti, 1998; Reid *et al.,* 1992). A potential mechanism for increased lipid damage in obesity seen in this study might be the increased lipid substrate within the myocardium that can serve as a larger target for oxidation by free radicals (Girotti, 1998; Lamers *et al.,* 1987). A negative consequence of the elevated myocardial oxygen consumption is the production of ROS such as superoixde, hydroxyl radical and hydrogen peroxides from the increased mitochondrial respiration. Indeed, leakage of electrons out of the mitochondrial electron transport chain promotes a one-electron reduction of molecular oxygen resulting in the formation of superoxide radicals (Turrens, 1997). The second mechanism by which obesity can independently cause increased lipid peroxidation is by progressive and cumulative cell injury observed histologically and resulting from pressure from the large body mass. Dandona *et al.,* (1998) have shown that cell injury causes the release of cytokines, especially tumor necrosis factor alpha (TNF-α) which generates ROS from the tissues, which inturn cause lipid peroxidation. A third possible mechanism to explain this observation is through the diet. Nutritional obesity which is the predominant form implies the consumption of

hyperlipidemic diets which may be involved in oxygen metabolism. Double bonds in the fatty acids molecules are vulnerable to oxidation reactions and may consequently cause lipid peroxidation. All the three mechanisms suggested above are operating in the obese of the present study. Recent evidence indicates that obesity is associated with increased myocardial lipid peroxidation and susceptibility to oxidative damage *in vitro* also (Vincent *et al.,* 1998). Investigators examining oxidative stress induced cellular injury can be lost due to oxidatively modified lipids and proteins (McDufee *et al.,* 1997). In the heart significant oxidative injury can ultimately lead to cardiac arthmias, poor contractibility, infraction, cardiac failure or sudden death (Yu, 1994). At least four general potential mechanisms could contribute to the obesity-induced myocardial lipid peroxidation a) increased myocardial work and subsequent radical production via mitochondrial respiration (Yu, 1994), b) decreased myocardial antioxidant defense (Desci *et al.,* 1997; Ohrvall *et al.,* 1993), c) increased fat deposition within myocardial tissue (Lamers *et al.,* 1987) and (or) d) increased rates of radical formation such as superoxide (O_2) or the hydroxyl radical (Braddy *et al.,* 1985). In the present investigation, obesity induction had caused an increase in lipid peroxidation and consequent free radical production and indicate low-antioxidant defense in heart. Further increased fat deposition within the myocardial tissue was also observed histologically leading to oxidative stress within the tissue. Previous reports indicate that lipid peroxidation is elevated in both liver and plasma in humans and obese animals (Desi *et al*., 1997; Koneru *et al.,* 1995). Furthermore, diets enriched in fats (either saturated or unsaturated) are also associated with increased lipid peroxidtion in several tissues such as aorta, liver and plasma (Folch *et al.,* 1951; Koneru *et al.,* 1995; Montilla *et al.,* 2006) reported that the cholesterol enriched diet caused clear increase in lipid peroxidation products in the brain kidney and erythrocytes.

SOD activity is undoubtedly important to the regulation of oxidative status in diabetes. Some studies have reported decreased SOD activity while others have shown increase in the enzyme activity in diabetic rats. Santiago *et al.,* (1993) showed an increase in SOD in the brain regions of aged rats (Hallivell and Gutteridge, 1999). There is strong evidence that the oxidative metabolism pathway of catecholamines exert their neurotoxic effect mainly due to the generation of highly reactive quinines and superoixde radicals. In the present study, the enzymatic activity of SOD was considerably decreased in the obese animals. The reason might be due to the increase in oxidative stress by obesity induction and probable utilization of the enzyme to scavenge H_2O_2 radicals. Increased lipid peroxidation might have led to cardiomyocytes damage and degeneration of cellular membranes and tissues as seen histologically. The two key enzymes SOD and CAT have been shown to decrease during the peak period of infraction due to peroxidiative insult as reported by Manjula *et al.* (1994). Administration of triphala and trikatu combination was more effective in restoring the obesity induced oxidative stress by eliminating excess superoxide or hydrogen peroxide ions. When compared to individual treatment of triphala and trikatu. Ayurslim, seemed to be more effective in its restorative action compared to other treatments. Catalase decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Hallivell and Gutteridge, 1999). In the present study, it was noted that catalase activity was lowered by induction of obesity at both durations of treatment. The decreased level of CAT activity observed may be due to the utilization of this enzyme in the removal of hydrogen peroxide radicals caused by obesity induction. Increased lipid peroxidation and extensive necrosis of cell membrane (seen in histology) is evidence to the oxidative stress of damaged myocardial tissue because of high fat diet.

Administration with combination of triphala and trikatu was effective in restoring the catalase activity. Both Ayurslim and Sibutrex10^{TM} had effectively restored the catalase activity to normalcy at both durations of treatment. These observations suggest the minimizing of necrosis in heart tissues by the herbs their cardioprotective properties. Glutathione (GSH) is an endogenous antioxidant in the human body. It may however delay the oxidation of LDL enabling it to leave the subsendothelial space before oxidation occurs. This may reduce the incorporation of LDL into the foam cells, thereby decreasing the atheroma development and progression. Under *in vivo* conditions, GSH acts as an antioxidant and its decrease was reported in diabetes mellitus by (Hallivell and Gutteridge, 1999). In the present study, it was noted that glutathione concentration was lowered by induction of obesity. The decrease in GSH levels represents increased utilization due to the oxidative stress. Administration with triphala, trikatu and their combination were moderately effective in restoring the glutathione concentrations to normalcy. Both ayurslim and Sibutrex10^{TM} were effective in restoring the glutathione concentration to normalcy at both the durations of their treatments. Triphala, being a mixture of *Terminalia chebula*, *Terminalia bellerica* and *Phyllanthus* emblica, has a greater reportoire of active components such as vitamin C, polyphenols tanins, saponins, carbohydrate derivatives, gallic and ellagic acids as well as anthroquinones. The extracts of *T.Chebula* in triphala exhibits negative inotropic and chronotropic effect on the heart muscle and hence recommended for heart diseases (Srivastava *et al.,* 1991). The rich ascorbic acid content of the herbs like amla extract inhibits radiation –induced lipid peroxidation in microsomes and SOD in mitochondria as shown by Khopde *et al.,* (2001). High anti-oxidant activity of trikatu might be due to the presence of capsanthin in pepper and gingerol in ginger both of them being its constituents.

The ethanolic extract of *Piper longum* (Pippali) fruits was found to protect mice against the radiation induced decline in WBC, bone marrow cells, α-esterase positive cells and GSH. Pippali extract also reduce the elevated levels of glutathione pyruvate transminase (GPT), alkaline phosphatase (ALP), and lipid peroxidation in liver and serum of irradiated animals (Sunila *et al*., 2005). Ginger has been reported to increase glutathione, reduce lipid peroxidation *in vivo* and scavenging various free radicals *in vitro* (Jagetia *et al.,* 2003; 2004). Effect of Ayurslim on lipid peroxidation might be due to the antioxidant activity of *T.chebula* and *Trigonella foenum* graecum. Earlier studies have shown that the protective effect of *T. chebula* fruit extracts against oxidative and peroxidative tissue damage might be attributed to its antioxidant potential.

Siburex10^{TM} shows weight reducing effect which in turn helped reduce oxidative stress.

Conclusion

The present study shows that obesity induction had caused an increase in lipid peroxidation and consequent decrease in superoxide dismutase, catalase and glutathione at both durations. Triphala, trikatu, and their combination were effective in restoring the above parameters. Ayurslim seems to be more effective in restoring lipid peroxidation and SOD activities. Siburex10^{TM} was effective in restoring catalase and glutathione. Our results suggest that the amelioration of obesity induced cardiotoxicity by Triphala and Trikatu may be related to its antioxidant property and therefore represents a potential therapeutic strategy for obesity With the exception of Orlistat and Sibutramine (Food and Drug Administration (FDA) approved drugs) most of the conventional drugs produce side effects and there is relapse of obesity after cessation of treatment. From this perspective botanicals have been screened for anti – obesity property. Triphala and Trikatu may be helpful in mitigating this particular side effect of such drugs. However, the effectiveness of the active components of Triphala and Trikatu in obesity and mechanisms involved require further investigation.

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