

ORIGINAL RESEARCH ARTICLE

Effect of Vitamin C, Vitamin E and superoxide Dismutase Mimetic *tempol* on Dexamethasone Induced Experimental Weight Loss In *wistar rats*.

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ABSTRACT

Dexamethasone is widely used Immunosuppressant and Anti-inflammatory agent. Its use is associated with evolution of Insulin Resistance and Hypertension and loss in body weight these are probably due to dysregulation of physiology. So in the present study, effect of Antioxidant like *Vitamin C* (Ascorbic acid), *Vitamin E* (α -Tocopherol) and SOD Mimetic *Tempol* (2,2,6,6,-tetramethyl-4-hydroxy-1-piperidinyloxy) was administered in the dose of (100mg/kg /day, mixed in water and food pallets were soaked in it, 100mg/kg /day mixed in sesame oil and food pallets were soaked in it, and 1mmol/L in drinking water respectively) and started from the initial day and continued throughout the study period for 14 days (Prophylactic regimen) and started from 8th day onwards the initiation of Dexamethasone and continued throughout the study period (Therapeutic regimen). Experimental Induced weight loss by using Dexamethasone (20 μ g/kg /once daily, subcutaneously) for 7 and 14 days in male Wister rats (240-300g) and estimated food intake, water intake, body weight daily and plasma total cholesterol at the last experiment day. From the finding of present study, *Vitamin C*, *Vitamin E* and SOD mimetic *Tempol* prevented /reversed the Dexamethasone induced decrease in body weight and prevent the decreased food intake only in preventive regimen. *Vitamin E* and *vitamin C* prevents decreased water intake in preventive and curative regimen respectively. There was no significant effect was observed in total cholesterol level in both the regimens.

Key Words: Dexamethasone, food intake, water intake, Body weight and Total cholesterol.

INTRODUCTION

Dexamethasone agent used for Immunosuppressive and Anti-inflammatory action. Its use induces adverse effects like Hypertension, Diabetes, Insulin Resistance and loss of body weight (Bjelakovic et al., 2007). Glucocorticoids have been shown to reduce cellular glucose uptake affecting the glucose transport system perse.¹ Thus Glucocorticoids have a role in the development of reduced body weight but the underlying mechanisms are still unclear. Hence, in the present study we aimed to evaluate the effect of different antioxidants like *Vitamin C*, *Vitamin E* and superoxide dismutase mimetic *Tempol* on Dexamethasone induced experimental weight loss in Wister rats to explore the role of food pattern.

MATERIALS AND METHODS

Male Wister rats weighing (240-300g) were used. They were housed in a group of six under environmentally controlled room with 12-h light/dark cycle and had free access to food and water. Dexamethasone injection was procured from Zydus cadila, *Vitamin C* (L-Ascorbic acid), *Vitamin E* (α -Tocopherol) was procured from Himedia Laboratories Ltd, Mumbai, India, *Tempol* (4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl freies RadiKal) was procured from Sigma Aidrich Germany. All the experimental procedures were carried out accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee (IAEC). Dosage of the

dexamethasone phosphate administered subcutaneously was prepared in the saline (0.9 % sodium chloride saline solution). Dose of the dexamethasone phosphate (20µg/kg b.w.) for the present study, were chosen based on previous reports.² *Tempol* was dissolved in drinking water (1mmol/l). Dose of the *Tempol* (1mmol/l) in drinking water for the study, were chosen based on previous reports.³ *Vitamin C* was mixed in water and food pallets were soaked in it. Dose of the *vitamin C* (100mg/kg body weight) through diet for the study, were chosen based on previous reports.⁴ *Vitamin E* was mixed in sesame oil and food pallets were soaked in it. Dose of the *vitamin E* (100mg/kg body weight) through diet for the study, were chosen based on previous reports.⁵ Instruments used for the study, Chem-5 Plus V2 Blood Chemistry – semi auto analyzer. (Erba Mannheim), Micro Centrifuge Model MC-2 RPM-10,000. (Genei Pvt. Ltd., Bangalore), Micro Pipettes (Erba Bio Medicals Ltd.) Weight loss was induced in rats by using dexamethasone by injecting dexamethasone 20µg/kg, subcutaneously once daily for 7 and 14 days. For the study animals were divided into 9 groups containing six in each as follows.

(1) Normal (NC): Received normal saline (0.9% NaCl) subcutaneously for 14 days.

(2) Dexamethasone (DEX-7): Received dexamethasone 20µg/kg subcutaneously, once daily for 7 days.

(3) Dexamethasone (DEX-14): Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days.

(4) Dexamethasone+ Tempol (DEX+TEMP): Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and treated with Tempol (1mmol) given in drinking water from 1st day and continued throughout study.

(5) Dexamethasone+ vitamin C (DEX+ Vit C): Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with chow mixed with Vitamin C (100 mg/kg bodyweight/day) from 1st day and continued throughout study.

(6) Dexamethasone+ vitamin E (DEX+ Vit E): Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with chow mixed with Vitamin E (100 mg/kg bodyweight/day) from 1st day and continued throughout study.

(7) Dexamethasone+ Tempol (DEX+TEMP). Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and treated

with Tempol (1mmol) given in drinking water from 8th day and continued throughout study.

(8) Dexamethasone+ vitamin C (DEX+ Vit C). Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with Vitamin C (100 mg/kg bodyweight/day) mixed in chow from 8th day and continued throughout study.

(9) Dexamethasone+ vitamin E (DEX+ Vit E). Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with Vitamin E (100 mg/kg bodyweight/day) mixed in chow from 8th day and continued throughout study. During the experimental period, Body weight, Food intake and Water intake was measured daily. At the end of treatment period, blood samples were collected by retro-orbital plexus puncture under light ether anesthesia after 16 h fasting (on 15th day) and blood was stored for biochemical estimation. Total cholesterol was estimated by using end point method by using a standard kit obtained from ERBA diagnostic Mannheim GmbH Ltd. (CHOD-PAP METHOD),⁶ results were showed in table no 1, 2, 3, 4, 5 and 6, and all data are presented as Mean ±SE. The statistical analyses were performed using unpaired student's t-test or One-Way ANOVA followed by Benfforroni multicomparison Test (post test). Statistical significance was assumed if $p < 0.05$.

RESULTS AND DISCUSSION

There was significant ($P < 0.0001$) decrease in body weight in rats injected with dexamethasone for 7 as well 14 days in rats injected with dexamethasone when compared with normal rats (**Table 1**). There was a significant decrease in water intake in rats injected with dexamethasone for 7 days ($p < 0.05$) as well 14 days ($p < 0.0001$). The average water intake was 32.07ml/day in rats injected with dexamethasone for 7 days, and it was 30.23ml/day in rats injected with dexamethasone for 14 days, where as in normal it was 35.20ml/day. There was a significant decrease in food intake in rats injected with dexamethasone for 7 days ($p < 0.001$) as well 14 days ($p < 0.0001$) when compared with normal rats. The average food intake was 11.63 and 11.17gm/day respectively, where as in normal it was 16.27gm/day. In rats injected with dexamethasone for 7 days, total cholesterol level was significantly ($P < 0.01$) decreased when compared with normal rats. Whereas, there was no significant difference in total cholesterol in rats

injected with dexamethasone for 14 days when compared with normal rats (**Table 2**).

Tempol, *vitamin C* *vitamin E* and significantly prevented the dexamethasone induced decrease in body weight ($P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively) when compared with dexamethasone injected rats (**Table 3**). *Vitamin E* significantly prevented the dexamethasone induced decrease in water intake when compared with dexamethasone injected rats. However, there was no significant difference in water intake was observed in rats treated with *tempol* and *vitamin C*. The average water intake in *tempol*, *vitamin C* and *vitamin E* treated rats it was 30.45 ml/day ($p > 0.05$), 30.28 ml/day ($p > 0.05$), 34.71 ml/day ($p < 0.001$) respectively, where as in dexamethasone injected rats it was 30.24 ml/day. *Tempol*, *vitamin C* and *vitamin E* significantly prevented the dexamethasone induced decrease in food intake. The average food intake in *tempol*, *vitamin C* and *vitamin E* treated rats it was 13.37 gm/day ($p < 0.01$), 15.19 gm/day ($p < 0.001$), 13.42 gm/day ($p < 0.01$) respectively. Whereas in dexamethasone injected rats it was 11.17. There was no significant effect was observed in total cholesterol when treated with *tempol*, *vitamin C* and *vitamin E* when compared with dexamethasone injected rats (**Table 4**). *Tempol*, *vitamin C* and *vitamin E* significantly reversed the dexamethasone induced decrease in body weight ($P < 0.01$, $P < 0.01$ and $P < 0.01$ respectively) when compared with dexamethasone injected rats (**Table 5**). *Vitamin C* significantly ($P < 0.001$) reversed the dexamethasone induced decrease in water intake when compared with dexamethasone injected rats. However, there was no significant difference in water intake in rats treated with *tempol* and *vitamin E*. The average water intake in *tempol*, *vitamin C* and *vitamin E* treated rats it was 29.03 ml/day ($P > 0.05$), 37.73 ml/day ($P < 0.001$) and 29.55 ml/day ($P > 0.001$) ml/day respectively, where as in dexamethasone injected rats it was 30.24 ml/day. *Vitamin C* significantly ($P < 0.001$) reversed the dexamethasone induced decrease in food intake. However, *tempol* and *vitamin E* had no effect on food intake. The average food intake in *tempol*, *vitamin C* and *vitamin E* treated rats it was 12.17 gm/day ($P > 0.05$), 14.77 gm/day ($P < 0.001$) and 12.91 gm/day ($P > 0.05$) respectively, whereas in dexamethasone injected rats was 11.17 gm/day. There was no significant effect was observed in total cholesterol level when treated with *tempol*, *vitamin C* and *vitamin E*

when compared to dexamethasone treated rats (**Table 6**).

Table 1: Gain in Body weight.

Groups	Treatment period(days)	% Change in body weight(grams)
Normal		+4.242 ± 0.9082
Dexamethason	7	-8.977 ± 0.777*
Dexamethason	14	-12.17 ± 0.5206*

Values are expressed in Mean ± SEM; n=6. (+) and (-) sign indicates gain and loss in body weight respectively, when compared to initial body weight. Statistical analysis was performed by using unpaired student's t-test. * $p < 0.0001$ when compared with normal.

Table 2: Plasma Total Cholesterol.

Groups	Treatment period(days)	Total cholesterol (mg/dl)
Normal		36.24±2.332
Dexamethasone	7	24.31±1.155**
Dexamethasone	14	41.47±14.370

Values are expressed in Mean ± SEM; n=6 rats per group. Comparison of mean values ween various groups were performed by unpaired student's t-test. Dexamethasone was administered subcutaneously for 7 day or 14 day. * $P < 0.05$ when compared with normal. ** $P < 0.01$ when compared with normal.

Table 3: Body weight gain. (Preventive effect of tempol, Vitamin C and Vitamin E).

Groups	% Change in body weight (grams)
DC	-12.17±0.5206
Tempol	-3.341±1.063**
Vitamin C	-2.805± 0.688**
Vitamin E	-5.472±0.608*

Values are expressed in Mean ± SEM; n=6. (+) and (-) sign indicates gain and loss in body weight. Statistical analysis was performed by One-way ANOVA followed by benferroni multi comparision Test. Dexamethasone-14 days Dexamethasone+Tempol(Tempol), Dexamethasone+Vitamin C (vitamin C), Dexamethasone+Vitamin E (vitamin E). DC and all treated groups were administered by subcutaneously for 14 days. * $P < 0.01$ when compared with DC. ** $P < 0.001$ when compared with DC.

Table 4: Plasma Total-cholesterol. (Preventive effect of tempol, Vitamin C and Vitamin E).

Groups	Total cholesterol (mg/dl)
DC	41.47±14.370
Tempol	27.98±3.675
vitamin C	26.12±3.627
vitamin E	15.385±4.457

Values are expressed in Mean ± SEM; n=6. Statistical analysis was performed by One-way ANOVA followed by benferroni multicomparision Test. Dexamethasone-14 days (DC), Dexamethasone+Tempol (Tempol), Dexamethasone+Vitamin C (vitamin C), Dexamethasone+Vitamin E (vitamin E).DC and all treated groups were administered by subcutaneously for 14 days. * $P < 0.01$ when compared with DC.

Table 5: Body weight gain. (Therapeutic effect of tempol, Vitamin C and Vitamin E).

Groups	% Change in body weight (grams)
DC	-12.17 ±0.5206
Tempol	-3.92 ±1.7778*
Vitamin C	-4.131 ±1.629*
Vitamin E	-4.81 ±0.9101*

Values are expressed in Mean ± SEM; n=6. (+) and (-) sign indicates gain and loss in body weight respectively, when compare to initial Body weight. Statistical analysis was performed by One-way ANOVA followed by benferroni multicomparision Test. Dexamethasone-14 days (DC), Dexamethasone+Tempol(Tempol), Dexamethasone+Vitamin C (vitamin C), Dexamethasone+Vitamin E (vitamin E). DC and all treated groups were administered by subcutaneously for 14 days. *P<0.01 when compared with DC. **P<0.001 when compared with DC.

Table 6: Plasma Total- Cholesterol. (Therapeutic effect of tempol, Vitamin C and Vitamin E)

Groups	Total cholesterol (mg/dl)
DC	41.47±14.370
Tempol	35.28±3.009
Vitamin C	23.52±5.279
Vitamin E	24.46±3.657

Values are expressed in Mean ± SEM; n=6. Statistical analysis was performed by One-way ANOVA followed by benferroni multicomparision Test. Dexamethasone-14 days (DC), Dexamethasone+Tempol(Tempol), Dexamethasone+Vitamin C (vitamin C), Dexamethasone+Vitamin E (vitamin E). DC and all treated groups were administered by subcutaneously for 14 days.

CONCLUSION

In conculsion, *Vitamin C*, *Vitamin E* and SOD mimetic *Tempol* Prevents/reverses the weight loss due to Dexamethasone induced weight loss in experimental model. All these Antioxidants in Prophylactic treatment only Prevents the reduced food intake in dexamethasone induced group. However, it not prevents the reduced food intake in Therapeutic regimen. Moreover it not alters Plasma total cholesterol level in prophylactic aswell therapeutic regimen. From the findings of present study, *Vitamin C*, *Vitamin E* and SOD mimetic *Tempol* Prevents/reverses weight loss in dexamethasone induced groups, here not only food pattern but also other factors are involved in Prevents/reverses weight loss in dexamethasone induced groups.

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