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Influence of Cold stress on STZ induced diabetic female rat organ weight: Anti-diabetogenic actions of phytoextracts

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Cold stress is considered as a co-morbid factor in diabetic subjects which causes reduced glucose tolerance and an increase in both corticosterone and glycaemia resulting in oxidative stress and damage of body tissues. This study aimed to address the changes in female reproductive organs somatic index in relation to glucose levels and feeding indices in diabetics exposed to cold stress and also evaluating the ameliorative efficacy of *Tribulus terrestris* fruit (TTF) and *Mesua ferrea* flower (MFF) extracts to tackle the insults.

Methods: Diabetic rats exposed to cold stress by housing in an acute cold stress apparatus at 4±2 °C for 3hrs per day for 7 days later they were supplemented with TTF and MFF extracts at a dose of 150, 200 and 250mg/kgbw and 100, 150 and 200mg/kgbw respectively. To check the efficacy of phytoextracts on glucose levels and on reproductive organs somatic indices, the animals were sacrificed and subjected to *in vivo* biochemical assays.

Results: The findings of this study confirm the synergistic effect of diabetes and cold stress on blood glucose levels and the deleterious effect on female reproductive organs weight viz, uterus, ovary and oviduct. Supplementation of TTF (200mg/kgbw) and MFF (150mg/kgbw) extracts found to be more effective on diabetic rats exposed to cold stress, the serum glucose levels decreased significantly ($P<0.05$) and significantly increased the weights of reproductive organs when compared with the intoxicated control groups.

Conclusion: Diabetes toxicity and cold stress in the female reproductive organs may result in disruption of oxidants and antioxidants balances, which provides a strong coupling of altered equilibrium processes and loss of energy capacity to meet an oxidation challenge and loss of organ tissues. Moreover, diabetics exposed to cold stress can increase the effects of oxidative stress. Exogenous supplementation of TTF and MFF extracts have been found to counter free radical generated oxidative stress and to facilitate reduction of the toxic effects induced by diabetes and cold stress, there by strengthening the cellular glucose tolerance and improved body and reproductive organs weight.

Keywords: Diabetes, Cold stress, Organ weight, *Tribulus terrestris* fruit, *Mesua ferrea* flower

INTRODUCTION

Streptozotocin (STZ), being diabetogenic agent is widely used to induce experimental diabetes in rodents as it destroys the pancreatic β-cells. In an experimental study on rats, streptozotocin given intraperitoneally at a dose of 65 mg/kgbw, effectively produced

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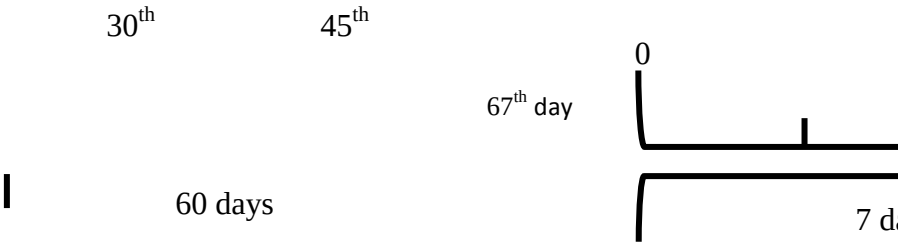
hyperglycemia and gastric mucosal ulcerations (Piyachaturawat *et al.*, 1988). Studies of Pouya Pournaghi *et al.*, (2012) advocated an augmentation in blood glucose levels in STZ induced diabetics that in turn demonstrated to affect various physiological systems including sexual function and fertility in women. Preponderance of studies relevant to exposure of cold stress confirmed decrease in body weight gain in animal models due to over stimulation of hypothalamic pituitary adrenal axis associated with low production of growth hormone (Alario *et al.*, 1987). Cold stress is considered as a co-morbid factor in diabetic subjects. For instance, Mondon *et al.*, (1963) studies advocated reduced glucose tolerance in diabetic rats upon exposure to cold stress (at 5 °C for 3 months), diabetic rats were unable to utilize a carbohydrate regimen effectively resulting in hyperglycemia. Studies suggested involvement of neuropeptide Y (NPY) in modulating the hyperglycemic effects in diabetic rats upon cold stress (at 4 °C for 2 hr/day for 14-days) wherein exacerbation of blood glucose levels were remarkable and caused early diabetic kidney disease. It is evident from the available literature that cold stress modulate(s) diabetic complications. However, available literature on female reproductive organs are very scare thereby this study intended to assess the effects of cold stress on diabetic rats. furthermore, the study intends to assess the ameliorative efficacy of *Tribulus terrestris* fruit and *Mesua ferrea* flower extracts as antidotes in modulating the cascade of effects connected to serum glucose levels and body weight. This study aimed to address the changes in preliminary aspects such as changes in OSI, especially female reproductive organs like uterus, ovary and oviduct in relation to food and feeding indices in diabetes and cold stressed subjects.

MATERIAL AND METHODS

Outline of the experimental design of the study and duration of cold stress exposure as well as regimen of phytoextracts supplementation are shown in below table. (The number of animals in each group comprises six female rats).



Tap water
STZ 45 mg/kgbw, ip, single dose
Cold stress (4±2 °C) 3 hr/day
STZ + Cold stress (4±2 °C) 3 hr/day

STZ + Cold stress (4±2 °C) 3 hr/day	TTF 150, 200 & 250 mg/kgbw/day	Group I Group II
STZ + Cold stress (4±2 °C) 3 hr/day	MFF 100, 150 & 200 mg/kgbw/day	Group III

Group IV

Group V (A,B,C)

GroupVI (A,B,C)

Feed and water consumption, body weight and organ somatic index: During experimentation, the rate of feed and water consumption (per day) recorded in all the groups and body weight (per week) too recorded. The organ somatic index (OSI) was calculated using the formula:

$$OSI = weight\ of\ the\ organ \times 100/weight\ of\ the\ body$$

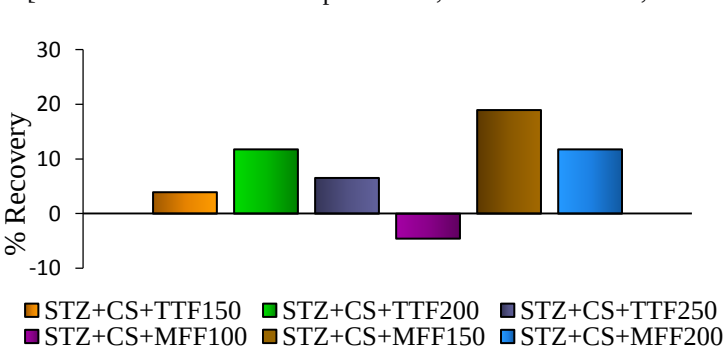
Estimation of Blood glucose levels: Fasting blood glucose levels were monitored by using ACCU-CHEK Active Glucometer (Ref- 07215762001) and the values are expressed as 'mg/dl'.

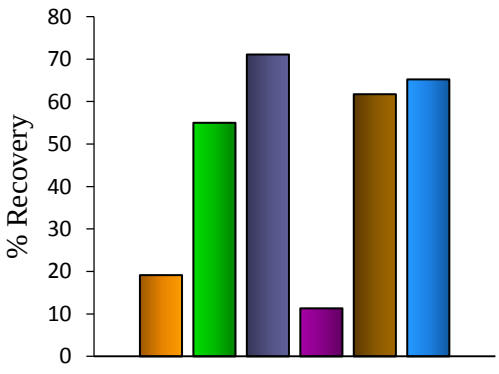
RESULTS

The data shown in Fig (1) depicts Increased feed and water consumption were observed upon STZ induction in rats while a decrease in feed and water intake rate was evident in cold stressed and positive control rats (STZ+ CS) (Table 1 and Fig 1). The phytoextracts (TTF and MFF) supplementation at 150, 200, 250 and 100, 150 and 200 mg/kgbw dose respectively caused considerable benefit by increasing feed and water intake consumption. Among the dose regimens tested with regard to both phytoextracts (TTF and MFF) only 200 and 150 mg/kgbw dose respectively exhibited significant effect in normalizing the rate of feed and water consumption.

(a) (b)

Figure 1. Dose-dependent influence of TTF and MFF on cold stressed diabetic rats: Percent (%) recovery observed with regard to rate of (a) feed and (b) water consumption.
[Abbreviations: STZ: Streptozotocin; CS: Cold stress; TTF:

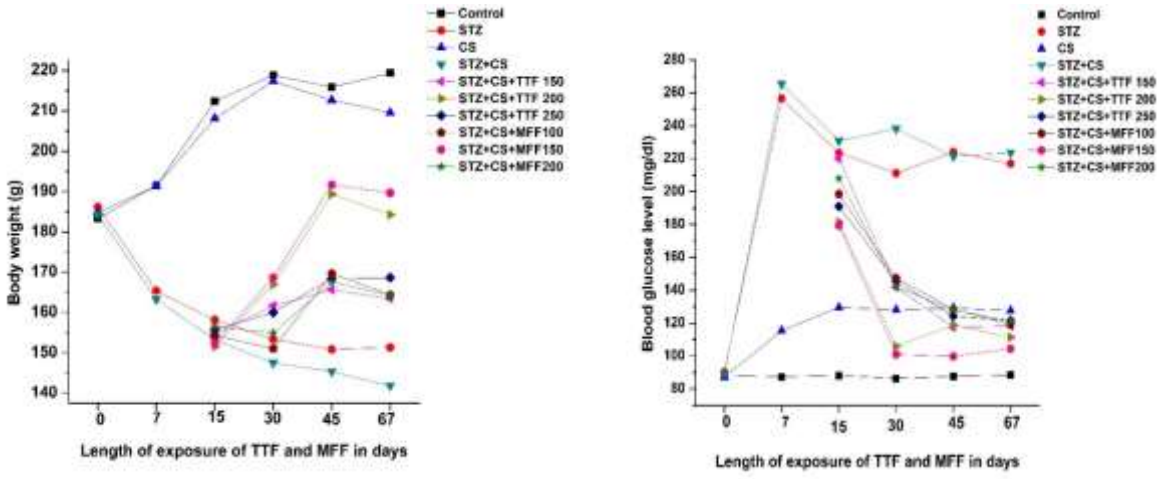




Tribulus terrestris fruit; MFF: *Mesua ferrea* flower and numerical 100, 150, 200 and 250 are dose(s) used in mg/kgbw as prophylactic treatment.]

In control group a steady state increase in body weight was observed while, in diabetic rats, cold exposure (4 °C) caused significant ($P<0.05$) decrease in body weight. In comparison, STZ diabetic and positive control rats (STZ+CS) were found to lose the body weight upon 30th day longevity of diabetic state and further decrease noticed on 45th day. Dissimilar pattern of results was evident in cold stressed rats, while supplementation of phytoextracts offered a slight recovery from 30th day of exposure to till 67th day. No significant beneficial effects were observed upon phytoextracts supplementation prior to 30th day of their exposure, while considerable amelioration in the form of recovery of body weight was evident from 30th day onwards. Among the doses supplemented, 200 (TTF) and 150 mg/kgbw (MFF)) doses were

found to be beneficial compared to other doses tested Fig (2a). It is evident from the results that diabetogenic agent STZ caused hyperglycemic state in experimental rats by destroying β-cells of pancreas. Likewise, prolonged exposure of cold stress to control rats caused hyperglycemic state from 8th day of its exposure to till the end. However, an exacerbation in hyperglycemic state was evident in diabetic rats upon cold stress. While supplementation of both phytoextracts (TTF and MFF) alone for two months (60-days) in various doses found to enunciate a significant ($P<0.05$) recovery in lowering the blood glucose levels in cold stressed as well as diabetic rats. In comparison, 19th and 15th day exposure of TTF and MFF at 200 and 150 mg/kgbw dose respectively were found to be effective in suppressing the hyperglycemic state Fig (2b).



(a) (b)

Figure 2(a). Dose-dependent influence of TTF and MFF on cold stressed diabetic rats: Percent (%) recovery observed with regard to OSI of (a) uterus (b) ovary and (c) oviduct.

Figure 2(b). Dose-response curves indicating modulatory effect of cold stress in diabetic rats and ameliorative changes in blood glucose levels upon exposure to TTF and MFF extracts.

[Abbreviations: STZ: Streptozotocin; CS: Cold stress; TTF: *Tribulus terrestris* fruit; MFF: *Mesua ferrea* flower and numerical 100, 150, 200 and 250 are dose(s) used in mg/kgbw as prophylactic treatment.]

A significant ($P<0.05$) decrease in OSI was observed with regard to female reproductive tract viz., uterus, ovary and oviduct in diabetic and cold stressed rats when compared to control.

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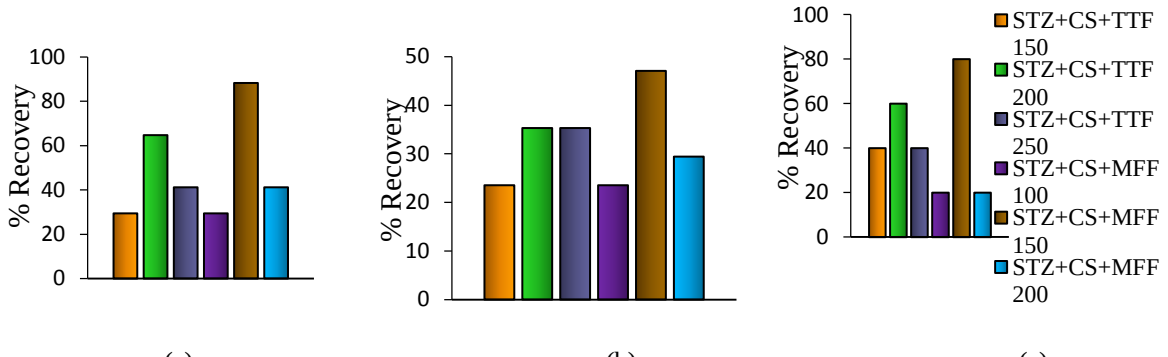
While in phytoextracts supplemented groups, OSI was found to marginally increased as a result of their TTF and MFF supplementation alone at a dose of 200 and 150 mg/kgbw respectively Fig (3).



(a) (b) (c)

Figure 3. Dose-dependent influence of TTF and MFF on cold stressed diabetic rats: Percent (%) recovery observed with regard to OSI of (a) uterus (b) ovary and (c) oviduct.
[Abbreviations: STZ: Streptozotocin; CS: Cold stress; TTF: *Tribulus terrestris* fruit; MFF: *Mesua ferrea* flower and numerical 100, 150, 200 and 250 are dose(s) used in mg/kgbw as prophylactic treatment.]

DISCUSSION

Several studies conducted earlier to assess the effect of diabetes and cold stress on different physiological systems along with its influence on feeding, water intake and body weight indicated diversified results. Assessments made on body weight can be considered as one of the important marker (s) which helps in predicting the extent of damage as a consequence of diabetes. In the present study a decrease found in body weight in cold stressed diabetic rats and similar decrease in body weight in diabetic models have already been reported by several researches (Salahuddin and Jalalpure, 2010) thereby the results of this study corroborates with the findings of earlier studies. Unlike the mean body weight the relative organ weight was found relatively high in cold stressed rats and results are in accordance with Kuo *et al*., (2007). Studies also confirmed similar alterations in uterine to body weight ratios consistent with the impaired ovarian steroidogenesis in diabetic rats and insulin deficiency in female rats resulting in low reproductive organ weight and elevated blood glucose levels (Cecillia *et al*., 1990). In this study severe diabetes led to decreased animal growth which could be due to catastrophic effects with the mediation of alterations in polyol pathway generally seen in diabetics (George, 2014). In the present study high blood glucose levels were evident in diabetic, cold stressed rats indicating diabetogenic actions of STZ and cold stress. In addition, cold stress induced exacerbation in glucose levels was evident in diabetic rats 'highlighting the modulatory effects of cold stress in altering the pathology. Similar observations were reported in earlier experimental studies (Eliasson, 1984). For instance, Eddouks *et al*., (2003) in their studies on STZ induced diabetic mice inferred that the fasting blood glucose levels as exhibited negative correlation with the body weight and positive correlation to feed and water consumption in STZ induced diabetic mice could accredit diabetogenic actions. Thereby, the results of present study are in accordance with aforesaid results. In the present study both normal and diabetic rats exposed to cold stress showed an increase in their blood glucose levels indicating diabetogenic actions of STZ as well as modulation of sympathetic tone

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resulting in hyperglycemic state in cold stressed animals. The present study results are in accordance with earlier reports of Caixeta *et al*., (2018), wherein chemically induced diabetes in addition to cold stress resulted in significant hyperglycaemia as well as decrease in body weight. In this study TTF and MFF supplementations at a dose of 200 and 150 mg/kgbw for 19 and 15-days respectively found to be effective in reducing blood glucose levels in experimental diabetic groups exposed to cold stress. The present study results are in accordance with Ercan and El, (2016), where TTF extract was shown to be very effective in decreasing hyperglycemic state by increasing insulin levels in STZ induced diabetic rats. Likewise, methanol extract of *Mesua ferrea* flower (200 mg/kgbw) has been shown to have promising antidiabetic activity in streptozotocin-induced diabetic rats and through increasing the secretion of insulin from pancreatic β-cells. In addition, the flower extract of *Mesua ferrea* also reduced the blood glucose levels and normalized the body weight in the diabetic rats compared to the untreated rats. In summary, *Tribulus terrestris* and *Mesua ferrea* extracts, having antioxidant potentiality showed significant antihyperglycaemic effect and possess the ability to recover the altered rate of feed and water consumption, blood glucose levels and body weight as well as organ somatic index.

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