ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021**

Cold Stress induced oxidative stress on diabetic female rat reproductive cycle: An Ameliorative effect of *Tribulus terrestris* fruit extract

Dakshayini P N, Mahaboob Basha P

Associate Professor, Department of Studies in Zoology, Maharani Cluster University
Palace Road, Bangalore-560001, INDIA.

ABSTRACT

Objective: The effects cold stress or diabetes caused pathophysiology are well known. This study aims not only to access the effects of cold stress but also its interaction with diabetes. The adverse effects of diabetes and cold stress on reproductive impairments is considered as a co-morbid factor for aggravating diabetic complications, since their co-exposure results in synergistic effects. The aim of this study was to address the extent of reproductive impairments occurred in co-exposed rats and the amelioration brought by ethanol extract of *Tribulus terrestris* fruit (TTF).

Methods: Female Wistar rats of induced diabetes (STZ 45mg/kgbw) were exposed to cold stress (4±2°C, 3hr/day for 7 days) and were supplemented with TTF extract (200 mg/kgbw/day for 21 days) to investigate the impaired reproductive performance.

Results: A significant change was evident in reproductive cyclicity among the positive control groups studied. Following hyperglycemic state, the estrous pattern and levels of reproductive hormones changed significantly ($P<0.05$) in both diabetic as well as in co-exposed groups. Further, supplementation of TTF extract was found beneficial in ameliorating the alterations induced upon co-exposure.

Conclusion: Synergistic effects of co-exposure witnessed in the functional tissues of female reproductive tract which found to disrupt the reproductive cyclicity by inhibiting estrogen and progesterone secretion. The polyphenol rich, TTF extract has the ability to quench free radical generated oxidative stress by enhancing the status of antioxidant enzymes thereby enhance endocrine functioning, to normalize reproductive ability in co-exposed rats.

Key words: Diabetes, Cold stress, *Tribulus terrestris,* reproductive cycle.

INTRODUCTION

Diabetes mellitus (DM), a endocrine and metabolic disorder, is initially characterized by loss of glucose homeostasis, resulting from defects in insulin secretion/action or both leading to impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins (Scheen,1997; American Diabetes Association, 2011). Moreover, as a consequence of hyperglycaemia, abnormally high levels of free radicals and decline of antioxidant defence systems have also been reported[3]. Chronic hyperglycaemia evokes oxidative stress by a variety of mechanisms including increased advanced glycation end-products formation, polyol pathway flux, glucose autoxidation and mitochondrial superoxide overproduction that eventually results in diabetes complications including reproductive disorders. DM induced oxidative stress which known to be involved in the normal human physiological functions

ISSN PRINT 2319 1775 Online 2320 7876

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

that could lead to the impairment of reproductive antioxidant defense mechanism. STZ induced diabetes, which causes severe insulinopenia, have revealed that both male and female rats with uncontrolled diabetes display a profound hypogonadotropic state, characterized by low basal levels of gonadotrophins and sex steroids, reduced luteinizing hormone (LH) pulsatility and defective gonadotrophin responses to gonadectomy. In diabetic females, disruption of positive-feedback effects of estradiol, delayed or absent preovulatory LH surges and anovulation are observed. Preponderance of studies have indicated that diabetes mellitus (DM) is found association with reproductive failure including delayed menarche, menstrual irregularities, and an increased incidence of infertility. Experimental studies using alloxan-induced diabetes in rats revealed delayed sexual maturity and an irregular estrous cycle, that resulted in reduced ovulation and reduction in the number of ova (Chieri *et al*., 1969). Similarly, earlier studies have demonstrated diminished LH surge in diabetic rats (Kirchick *et al*., 1978; Vomachka *et al*., 1982).

Stress as an adaptive response of the body, produces a wide range of biochemical and behavioural manifestations to respond to a threat and is known to alter the physiological homeostasis of the organism and complex mechanisms contributing to the breakdown in adaptation processes. Cold stress increased ovarian norepinephrine (NE) levels and induced ovarian function alterations, which led to polycystic ovarian morphology in rats and also induced the formation of follicular cysts and follicles with hyper- thecosis alongside increased plasma estradiol and testosterone levels, irregular estrous cyclicity, and reduced ovulation.

Plants often contain substantial amounts of antioxidants, and suggest that antioxidant action may be an important property of plant medicines associated with diabetes. The increasing interest in various medicinal plants and their bioactive compounds has led to increased attention to their safety and efficacy in the treatment of diabetes and also alternative treatment of female reproductive disorders using herbal remedies is gaining popularity in human medicine. *Tribulus terrestris* L. (TT) from Zygophyllacea family, is an annual herb that grows worldwide, especially in the subtropical regions, and is used in traditional medicines in India, China, South Africa, Bulgaria and other countries against sexual impotency, edema, abdominal distention, and cardiovascular diseases. In modern pharmacological studies, reported that *Tribulus terrstris* fruit (TTF) ethanol extract was found to be effective in treating streptozotocin-induced hyperglycemia[24,25]. Further studies also disclosed that TT improved reproductive function, including increased concentration of hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol, with significant effect on both ovarian and uterine activities, thereby improving reproductive performance, libido and ovulation. In light of this, the study was designed to investigate the effectiveness of TTF ethanol extract in STZ-induced diabetic female rat reproductive system exposed to cold stress and to evaluate their therapeutic potential for the treatment of cold stress induced modulations on antioxidant status of DM.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

MATERIALS AND METHODS

Induction of experimental diabetes mellitus- Diabetes mellitus was induced in rats by the intraperitoneal injection (volume of 1ml/kg body weight) of freshly prepared streptozotocin (STZ) at a dose of 45 mg/kg body weight dissolved in 0.1 M citrate buffer solution of pH 4.5. Three days after the STZ injection, the blood was withdrawn from the tail vein, and the glucose level was determined. Rats were diabetic when their fasting blood glucose levels were more than 200 mg/dL.

Induction of cold stress- To induce cold stress, rats were housed in an acute cold stress apparatus (Colton BOD incubator) at 4±2 °C for 3hrs per day for 7 days on a 12-h light/12-h dark cycle with a built-in heater and cooler that could be controlled by self-timer.

Estrous cycles were monitored by colpo-cytological examination (vaginal smears) daily for 30 consecutive days (four weeks). Cells detaching from the vaginal epithelium were removed with a pipettes. Filter tips containing 10 µl 0.9% saline were discarded after the vaginal secretions had been transferred to clean slides (Marcondes et al., 2002). Colpo-cytological examination time was set at 09.00 hours. Each slide was analysed under a microscope (Labomed ATC 2000) at 10x and 20x magnifications. Only female rats showing two consecutive estrous cycles of the same length were used for experimentation (Iranloye and Bolarinwa, 2007). The length of estrous cycle and number of cycles per month were recorded in both control and test animals.

Supplementation of extract- Among the examined doses through pilot study (50-250mg/kg bw/day), the most effective dose of TTF extract was 200mg/kg, to check the efficacy of TTF extract on oxidative stress indices in uterus, ovary and oviduct, the animals were sacrificed after 19 days of TTF exposure as the extract has shown anti-hyperglycemic effect on the 19th day of TTF extract exposure.

Experimental Design- Rats were divided into seven groups: Group I— control animal group was kept at the laboratory room temperature; Group II—induced diabetes; Group III—exposed to cold stress at 4±2 ºC; Group IV—diabetic rats exposed to cold stress at 4±2 ºC; Group V—induced diabetes plus supplemented with TTF; Group VI—exposed to cold stress at 4±2 ºC plus supplemented with TTF; Group VII —diabetic rats exposed to cold stress at 4±2 ºC plus supplemented with TTF. Dissected reproductive organs viz uterus, ovary and oviduct tissues were washed in ice-cold saline, patted dry. The tissue homogenates were made by using appropriate buffer and supernatant was stored at a temperature of -20 ºC and used for biochemical assays.

STATISTICAL ANALYSIS

The results are expressed as mean ± SD of six observations (n = 6) in each group. Differences between treatment groups were assessed by one-way analysis of variance (ANOVA) using the SPSS software package for windows version 20.0. Post hoc testing was for inter-group comparisons using Bonferroni test at probability (P) value 0.05 level of significance.

RESULTS

The findings of this study confirm the deleterious effect of diabetes and cold stress on the body and organs weight, antioxidant defense system and hormones of female reproductive organs viz uterus, ovary and oviduct.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021**

Supplementation of TTF extract to diabetic rats, the serum glucose levels decreased significantly ($P<0.05$) on the 19th day of extract administration and further remained constant till 30th days of TTF exposure, indicating the anti-hyperglycemic properties of the fruit extract of TT, as the substance with anti-hyperglycemic properties would be effective in the management of diabetes (Table 1). Statistical analysis reveal that the serum glucose levels reduced at a dose of 200mg/kg body weight suggesting the ameliorative role of TTF on the 19th day of exposure in extending protection to diabetic animals, compared to other doses. Hence, the present study demonstrates that TTF extract of 200mg/kg body weight dosage was found to be an effective dose.

***In vivo* Biochemical assays**

In this study, diabetes and cold stress co-exposure induced decrement in SOD activity was evident in all the functional tissues; however, ovary was severely affected than other tissues studied, showed the greatest decrease in activity (-89.7%). The one-way ANOVA indicated an interaction between diabetes-induced toxicity and cold stress resulting in a significant decrease in SOD levels. In general diabetes and cold stress alone also significantly decreased the SOD levels in all the functional tissues studied (Fig. 1).

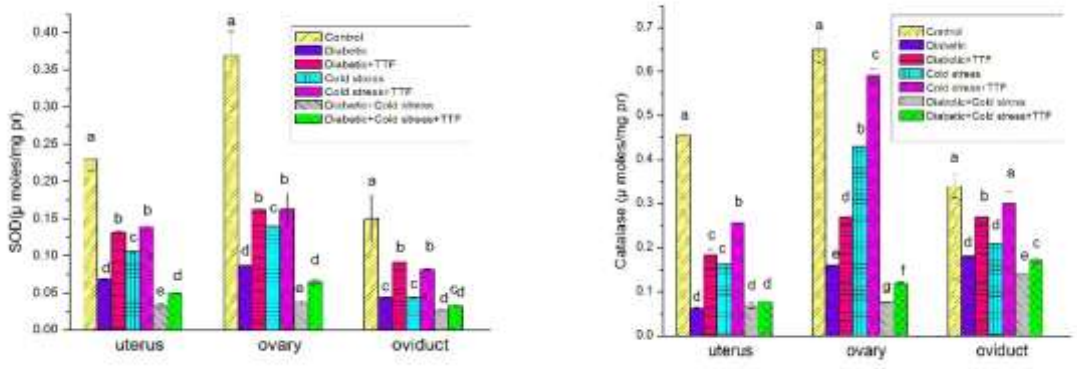

Fig.1: Effect of *Tribulus terrestris* fruit ethanol extract on SOD and Catalase (Cat) antioxidant enzyme activity levels in reproductive system (uterus, ovary & oviduct) of STZ induced diabetic female rats exposed to cold stress (4±2 °C). Values are mean ±SD of six observations and a,b,c,d…..letters denote significantly different from control values by one-way ANOVA($P<0.05$).

There was a marked decrease in CAT activity as a consequence of severe diabetes. Among the three organs studied in the female rats, the uterus showed the greatest decrease in the activity of CAT (-86.0%) and cold stress treatment at low temperature also significantly decreased CAT levels in uterus while the changes were mild in ovarian and oviduct tissues. The one-way ANOVA indicated an interaction between diabetes toxicity and cold stress treatment performed at low temperature resulting in a significant decrease in CAT levels in all functional tissues studied (Fig.1).

Dietary antioxidant supplementation proved to be effective in restoring oxidative damage evidenced by diminishing elevated MDA levels and enhancing the inhibited activities of SOD and CAT expressed in terms of % recovery against enzyme activities induced by diabetes and

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021**

cold stress. In uterus, the minimum ameliorative effect of supplementation was observed with TTF extract on the enzyme activities maximum with CAT (+190.4%) and followed by SOD (+91.3%), in diabetic group. The maximum ameliorative effect of supplementation was observed with TTF extract on an enzyme activities moderate with CAT (+57.0%) and minimum activities with SOD (+31.1%) in cold stressed group.

The minimum ameliorative effect of supplementation was witnessed with TTF extract on an enzyme activities maximum with SOD (+54.5%) in co-exposed group. In ovary, the minimum amelioration occurred with the supplementation of TTF on the enzyme activities with SOD (+68.7%) and CAT (+57.0%) in diabetic group. The moderate amelioration occurred with the supplementation of TTF on the enzyme activities with CAT (+37.2%) and SOD (+16.4%) in cold stressed group.

The moderate amelioration occurred with the supplementation of TTF on the SOD (+71.0%) and CAT (+53.8%). In the oviduct, moderate amelioration occurred with TTF on the enzyme activities on SOD (+106.8%) and CAT (+50%) in diabetic group. In the oviduct, the moderate amelioration occurred with TTF on the maximum enzyme activities on SOD (+86.3%), and minimum enzyme activities on CAT (+42.8%) in cold stressed group. In the oviduct, minimum amelioration occurred with TTF on the enzyme activities SOD (+22.2%) and CAT (+21.4%) in co-exposed group.

There was a significant difference in estrous cyclicity among the three positive control groups studied when compared to control. Following hyperglycemic state, the estrous pattern changed significantly ($P<0.05$) in both STZ-induced diabetic as well as in co-exposed groups (STZ+CS). In comparison, the frequency of estrus phase was significantly reduced ($P < 0.05$) and the animal remain in di-estrous and pro-estrous phase for long duration in both STZ-induced diabetic as well as in co-exposed rats (Fig 2). Wherein, estrous smears occurred with considerable irregularity and there was more significant ($P<0.05$) increase in the duration of estrous cycle and decrements in number of cycles per month in STZ-induced diabetic as well as in co-exposed rats. To explicit the results in brief, the normal 4 or 5-day pattern was replaced by 6 to 7-days and number of cycles per month was slightly altered and there were slight changes in the duration of each phase of estrous cycle in cold exposed rats when compared to control. Furthermore, it is evident from the results that the supplementation of both phytoextracts, TTF and MFF alone at a dose of 200 and 150 mg/kgbw respectively was found beneficial in ameliorating the alterations induced upon co-exposure of diabetes and cold stress. In comparison, MFF at a dose of 150 mg/kgbw was found beneficial than TTF.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021

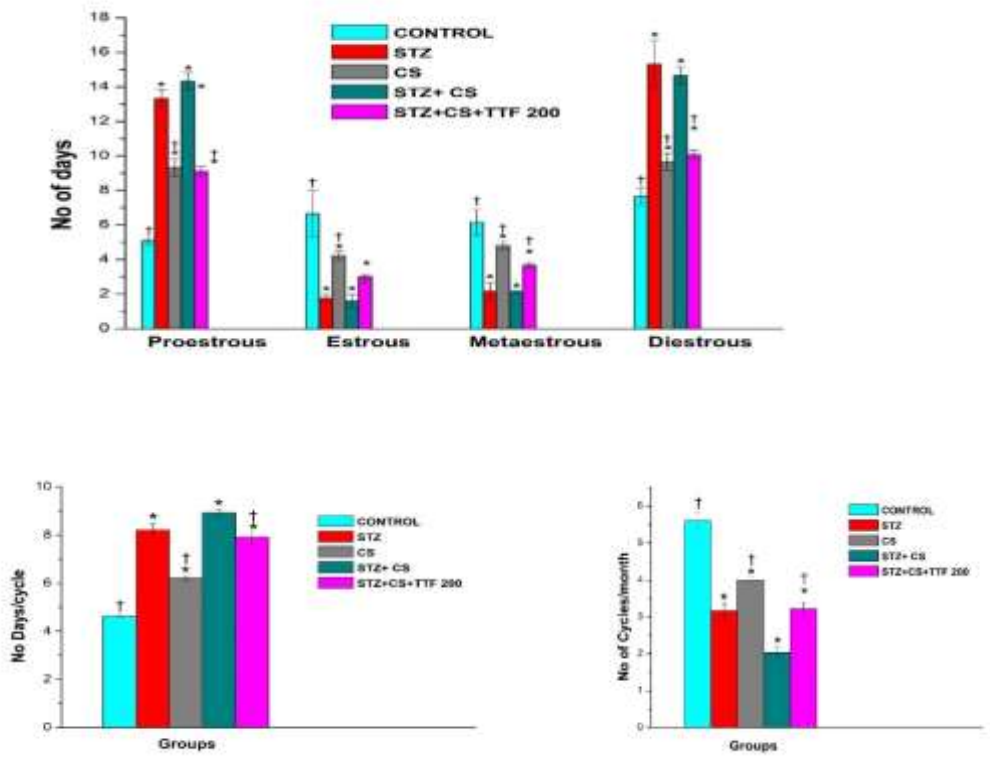

Fig. 2: Ameliorative effect of Tribulus terrestris fruit extract on and (a) estrous pattern, (b) number of days /cycle and (c) number of cycles/month in STZ induced diabetic rats exposed to cold stress(4±2ºC). Values are means ±SEM of six animals, *($p<0.05$) represents significant difference vs. control & †($p<0.05$) represents significant difference vs. positive control(STZ) groups

DISCUSSION

Increased glucose-oxidation, non-enzymatic glycation of proteins and their subsequent degradation cause unbalanced free-radical generation in diabetes Indeed, hyperglycaemia mediated advanced glycation of intracellular antioxidant defence enzymes results in hyper-susceptibility to the elevated oxidative stress due to lowered anti-oxidative protection. In the present study the elevated glucose level in all the experimental rats was significantly lowered upon TTF supplementation and may be through inhibition of α-glucosidase as well as by its antidiabetic effects and the results are in accordance with Lamba et al. Our results indicated a decrease in body and reproductive organs weight of the diabetic and co-exposed rats in comparison to the normal control rats. The decrease in body and reproductive organs weight was as a result of loss of tissue proteins and muscle mass in diabetes. In this study, supplementation of TTF extract at a 200mg/kg bw dosage level in STZ-induced diabetes intoxicated and co-exposed rats produced a significant protective effect against reproductive organs functional tissues toxicity and this effect characterized by increased weights of body and reproductive organs viz., uterus, ovary and oviduct, improved follicular quality and quantity. These findings are in accordance with those previously reported.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021**

Elevated systemic glucose level promotes overproduction of superoxide radical (O_2•-) and H_2O_2 and ROS are associated as important physiological and pathological mediators in many reproductive disorders. ROS is involved in the modulation of an entire spectrum of physiological reproductive functions such as oocyte maturation, ovarian steroidogenesis, corpus luteal function and luteolysis. The altered balance of the antioxidant enzymes with a decrease in SOD and CAT activities in STZ diabetic and cold stressed condition, may be due to increased production of superoxide and H_2O_2 by the auto-oxidation of the glucose and non-enzymatic glycation and this depicts the inactivation of the enzymes by superoxide anions.

The mechanism(s) underlying the protective effect of TTF against reproductive toxicity induced by diabetes and cold stress in rats could be attributed to its potent anti-oxidant property, so reducing oxidative stress in the reproductive organs and improving reproductive function by increased release of estradiol, FSH and LH serum levels. Estrous cycle in rat involves many histological, physiological and morphological as well as biochemical changes within the ovary, wherein during estrous cycle maturation and ovulation of pre-ovulatory follicles takes place under the influence of ovarian and extra-ovarian hormones (Smith *et al*., 1987). Any imbalance in surge of hormones leads to irregularity in the function of ovary and bring changes in the duration of estrous cycle (Koneri *et al*., 2006). Further, Oxidative stress also found to cause damage during oocyte maturation and ovulation (Agarwal *et al*., 2005). In this study, estrous cycle exhibited normal 4 or 5-day pattern, while 6 or 7-days estrous cyclicity per month was observed in cold stressed rats. Similar results were pronounced by Saraswathi *et al*., (2012, 2010) wherein, cold restraint stressed rats showed a significant increase in the mean number of days in proestrous phase and decrease in estrous and metestrous phases indicating arrest of follicular development at initial stages leading to non-maturation of follicles.

In 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. Moreover, exposure to diabetes and cold stress can increase the effects of oxidative stress. Exogenous supplementation of TTF extract has 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 antioxidant defense and improved reproductive functioning ability.

ACKNOWLEDGEMENTS

This study was supported by UGC-SWRO, Bangalore in the form of Faculty Improvement Programme (FIP) to Dakshayini P N.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021**

REFERENCES

1. International Diabetes Federation, *Diabetes Atlas*. (Brussels, Belgium), 2013, 6th Edition.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus, *Diabetes Care*, 34(Suppl 1) (2011) 62.
3. Maritim AC, Sanders RA & Watkins JB. Diabetes, oxidative stress, and antioxidants: A Review. *Journal of biochemical and molecular toxicology*, 17 (2003) 24.
4. Katayama S, Brownscheidle CM, Wootten V, Lee JB & Shimaoka K, Absent or delayed preovulatory luteinizing hormone surge in experimental diabetes mellitus, *Diabetes*, 33 (1984) 324.
5. Dong Q, Lazarus RM, Wong LS, Vellios M, Handelsman DJ & Pulsatile LH, Secretion in streptozotocin-induced diabetes in the rat, *J Endocrinol*, 131 (1991) 49.
6. Valdes CT, Elkind-Hirsch KE, Rogers DG & Adelman JP, The hypothalamic-pituitary axis of streptozotocin-induced diabetic female rats is not normalized by estradiol replacement, *Endocrinology*, 128 (1991) 433.
7. Kienast SG, Fadden C & Steger RW, Streptozotocin-induced diabetes blocks the positive feedback release of luteinizing hormone in the female rat, *Brain Res Bull*, 32 (1993) 399.
8. Steger RW & Rabe MB, The effect of diabetes mellitus on endocrine and reproductive function, *Proc Soc Exp Biol Med*, 214 (1997) 1.
9. Chrousos GP, Stressors, stress, and neuroendocrine integration of the adaptive response, The 1997 Hans Selye Memorial lecture. *Annals of the New York Academy of Sciences*, 851 (1998) 311.
10. Szabo S, Hans Selye and the development of the stress concept: special reference to gastroduodenal ulcerogenesis, *Annals of the New York Academy of Sciences*, 851 (1998) 19.
11. Tache Y & Brunnhuber S, From Hans Selye's Discovery of biological stress to the identification of corticotropin-releasing factor signalling pathways: Implication in stress-related functional bowel diseases, *Annals of the New York Academy of Sciences*, 1148 (2008) 29.
12. Chrouses GP, Philip W & Gold MD, The concept of stress system disorders, *JAMA*, 267(1992) 1244.
13. Goel RK & Bhattacharya SK, Gastroduodenal mucosal defense and mucosal protective agents, *Indian J. Exp. Bio*, 29 (1991) 71.
14. Gotoh M, Tajima T, Suzuki Y, Ikari H, Iguchi A, Kakumu S & Hirooka Y, Swimming stress that causes hyperglycemia increases in vivo release of noradrenaline, but not acetylcholine, from the hypothalamus of conscious rats, *Brain Research*, *780*(1) (1998) 74.
15. Angelogianni P & Gianoulakis C, Ontogeny of the beta-endorphin response to stress in the rat: Role of the pituitary and the hypothalamus, *Neuroendocrinology*, 50 (1989) 372.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** **UGC CARE Listed (Group -I) Journal Volume 10, Iss 1, 2021**

16. Smythe GA, Grunstein HS, Bradshaw J E, Nicholson MV & Compton PJ, Relationships between brain noradrenergic activity and blood glucose, *Nature*, 308 (1984) 65.
17. Smythe GA, Pascoe WS & Storlien LH, Hypothalamic noradrenergic and sympathoadrenal control of glycemia after stress, *Am. J. Physiol*, 256 (1989) E231.
18. Dhanalakshmi S, Srikumar R, Manikandan S, Parthasarathy NJ & Devi RS, Antioxidant property of triphala on cold stress induced oxidative stress in experimental rats. *J Health Sci*, 52 (2006) 843.
19. Selye H, The general adaptation syndrome and the diseases of adaptation, *J. Clin. Endocrinol*, 6 (1946) 117.
20. Paredes A, Galvez A, Leyton V, Aravena G, Fiedler JL, Bustamante D & Lara HE, Stress promotes development of ovarian cysts in rats: The possible role of sympathetic nerve activation, *Endocrine*, 8 (1998) 309.
21. Dorfman M, Arancibia S, Fiedler JL & Lara HE, Chronic intermittent cold stress activates ovarian sympathetic nerves and modifies ovarian follicular development in the rat, *Biology of reproduction*, 68 (2003) 2038.
22. Greiner M, Paredes A, Araya V & Lara HE, Role of stress and sympathetic innervation in the development of polycystic ovary syndrome, *Endocrine*, 28 (2005) 319.
23. Bernuci MP, Szawka RE, Helena CV, Leite CM, Lara HE & Anselmo-Franci JA, Locus coeruleus mediates cold stress-induced polycystic ovary in rats, *Endocrinology*, *149*(6) (2008) 2907.