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Evaluation of therapeutic effects of aqueous cinnamon extract against obesity associated dysregulation of pituitary-adrenal axis

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***Abstract*---**Obesity is distinguished by unwanted and surplus accumulation of lipids in body principally in adipocytes. Inactive lifestyle and dietary irregularities are considered as premiers among a variety of obesogenic risk factors. Obesity is associated with variations in the concentration of trophic and tropic hormones from pituitary gland affecting different body parts and other endocrine glands including adrenal gland. Current study was design to explore the anti-obesity potential of cinnamon (*Cinnamomum verum)* against high sugar and high fat induced hyper-activity of pituitary-adrenal (PA) axis and obesity. Twenty-five healthy Wistar albino female rats were selected as model animal. Body weight, oral glucose tolerance test, serum cortisol, hematology and histopathological analysis were performed. The high sugar and high fat induced increase in body eight was decline by cinnamon administration. Hematological analysis revealed a significant decreased concentration of RBCs and hemoglobin and increased WBCs and platelet count following sub-acute exposure of high sugar and high fat changes were attenuated by cinnamon. High sugar and fat associated elevated serum cortisol was normalized after cinnamon exposure. Moreover, cinnamon protected the histopathological alterations following high sugar and fat administration, showed protective effects of Cinnamon against obesity associated dysregulation of PA axis and metabolic impairment.

***Keywords*---**Obesity, Metabolic syndrome, Cortisol, Cinnamon.

Introduction

Obesity is described by unwanted and disproportionate accumulation of lipids in the body mainly in adipose tissue. Obesity has ranked among principle causes of preventable death. Obesity is now considered as an epidemic due to its swift incidence for the past several years (Tomiyama *et al.*, 2018). Substantial increase in obesity prevalence has been observed during the last 4 decades. According to statistics from 1975 to 2016, highest rate of incidence of obesity was noted in women with an increase from 6% to 15%, lowest in men with an increase from 3% to 11% while an intermediate rate was observed among boys and girls with an increase from 1% to 8 % (Jaacks *et al.,* 2019). A number of experimental investigations and World Health Organization (WHO) has reported exponential growth of childhood obesity as well. They have indicated that about 0.340 billion children and youth with ages ranging between five to nineteen years were either overweight or obese in 2016. Of them, 124 million were classified as obese (Chakraborty *et al.,* 2019). Existing literature has indicated obesity induced improper regulation of pituitary-adrenal axis through Hyperactivation of axis (Hunter and Syed, 2018: Incollingo Rodriguez *et al.*, 2015). Secretion of ACTH

(Adrenocorticotrotrpoic hormone) from pituitary increases that ultimately results in high serum Cortisol level. Long term exposure of Cortisol enhances desire for food especially those enriched with sugars or fats with reduction in activity creating an imbalance between intake and actual body requirement, causing over building of energy reserves (Hewagalamulage *et al.*, 2016).

Pituitary gland being the master gland of body influences a variety of activities either directly or indirectly by regulating the secretions of other endocrine glands including adrenal gland. Pituitary hormones are essential for a number of vital body processes like growth, homeostasis, reproduction, stress response and metabolism. Each hormone is released in a pulsating way and carefully regulated via complex interactions of feedback pathways (Hong, Payne, and Jane, 2016: Samarasinghe, *et al.,* 2014). Pituitary secretes ACTH that via circulation targets its target sites in adrenal gland to stimulate release of Cortisol (Emerald, 2016).

Cinnamon is a spice that belongs to *Lauraceae* family and is famous for its flavoring characteristics. Bark of cinnamon has been used throughout the world especially in china for centuries to treat a number of complications. A variety of investigations have noticed therapeutic effects of cinnamon against bacteria, virus, fungi, cancer, oxidants and hypertension (Hajimonfarednejad *et al.*, 2018;Gruenwald *et al.,* 2010). Moreover, it has good protective potential against metabolic syndromes including diabetes and obesity (Mollazadeh and Hosseinzadeh, 2016) by lowering serum Cholesterol level and increasing insulin sensitivity (Anderson *et al.*, 2016). It has also been proposed that cinnamon demonstrates therapeutic power against obesity by inducing browning of white adipose tissue (Zuo *et al.*, 2017). Cinnamon does not contain many vitamins or minerals, but it is loaded with antioxidants that tend to minimize oxidative stress resulted by hypercorticism in obesity. Cinnamon taken in the form of tea has resulted significant weight loss. The major role is played by Cinnamaldehyde, one of several ingredients of Cinnamon that activates fat cells to start burning energy (Gowri *et al.,* 2017).

In view of previous literature, current study will focus on the physiological alterations in PA axis resulted by high sugar and high fat diet induced obesity. Then extent of therapeutic potential of Cinnamon was investigated by measuring plasma concentrations of Cortisol hormone.

Materials and Methods

Animals

Twenty-five healthy albino female rats aged one month weighing about 150-250 g were kept in a well-ventilated animal house under standard housing conditions in the animal room of Institute of Physiology and Pharmacology, University of Agriculture.

Experimental Material

The materials used for experiment were commonly used food products including blue band margarine as a source of fat and sucrose (table sugar) as a source of

sugar in a 30:70 ratios with routine chow diet to induce obesity. Blue band margarine composed of polyunsaturated fatty acids and trans-fats while sucrose contains simple sugars (glucose), both being rich source of high calories. Cinnamon extract, the treatment entity along with obesogenic materials (margarine and sucrose) were purchased from a departmental and grocery store in Faisalabad. The dose of cinnamon was calculated according to the body weight of rats (200 mg/kg). The calculated dose was administered by intragastrically with oral gavage.

Cinnamon extract preparation

In order to prepare extract, Cinnamon bark was dried and grinded into fine powder using electrical grinder. A quantity of 200 g of powder was dissolved in 1000 ml of distilled water and boiled for approximately 10 minutes. At last it was cooled down and filtered. Freshly prepared aqueous extract was stored at room temperature for gradual use.

Study design

All the experimental animals were separated into five equal groups. First group was considered as control and fed only with routine feed and water until the completion of trial. Second group was taken as high sugar (HS) and received 30% sucrose mixed with 70% of routine diet. Third group was taken as high sugar + Cinnamon (HS+C) and received aqueous Cinnamon extract (200 mg/kg/day) along with high sugar diet. Fourth group was high fat (HF) group and fed on 30% fats mixed with 70% of normal diet. Fifth group was high fat + Cinnamon (HF+C) and received Cinnamon (200 mg/kg/day) in addition to fat rich diet for 28 days.

OGTT Analysis

As an attempt to access insulin insensitivity, oral glucose tolerance test was performed on 28th day of trial. Blood and organ samples were collected at 28th day from all of the 5 groups for complete blood count and serum cortisol hormone analysis.

Serum Hormonal Analysis

The blood samples collected in gel clot activator vacutainers were centrifuged at a rate of 4000 revolutions per minute for 10 minutes to split serum. Serum was further subjected to cortisol analysis.

Cortisol

ELISA was performed in order to determine cortisol level by commercially available cortisol ELISA KIT, catalog No. 10017C BIOS. It's a type of competitive ELISA in which microwells of ELISA plate are coated with streptavidin. Described quantity of serum samples to be evaluated is mixed with working cortisol HPR conjugate and anti-cortisol biotin solution. Concentration of cortisol in the sample competes with the cortisol enzyme conjugate (HRP) for the attachment with binding sites. Procedure:1) Pipetted 25 µl of cortisol standard, controls and

samples to be tested into appropriate microtiter wells.2) Added a quantity of 50 µl of biotin reagent into all wells where required. 3) Then added a concentration of 100 µl of cortisol enzyme conjugate to all the wells.4) Used vortex mixer to thoroughly mix all added constituents for 10 seconds.5) Incubated for a period of 1 hour at room temperature (20-25 ℃).6) After carefully removing liquid from all wells, all the wells were washed properly thrice with a volume of 300 µl of 1X wash buffer and then blotted on an absorbent paper towel. 7) Then a volume of 100 µl of TMB substrate was dispensed to all wells where required.8) Set to incubate for a period of fifteen minutes at room temperature of 25 ℃.9) At last, a volume of 50 µl of stop solution was dispensed to all wells.10) Gently taped the plate and mix the solution properly. 11) Finally, absorbance of all wells was noted with the help of ELISA reader. Absorbance was taken 15 minutes later at a wavelength of 450 nm, after dispensing of stop solution and following curve was developed from standards and quantity of unknown samples were calculated from the equation.

Hematological analysis

All the animals were slaughtered carefully and blood samples were collected. A cut was made at left side of neck in order to collect samples from jugular vein. First 02 ml of blood was collected in EDTA tubes for CBC test while remaining was collected in gel clot activator for hormonal analysis. On same day of sample collection, sample were referred to hematology lab for complete blood count analysis. CBCs analysis was carried out on SemiAutomatic Haematology Analyzer Mindray CBC Machine.

Histological Analysis

Rats were slaughtered to collect their pituitary and adrenal glands for histological examination. The standard procedure for histological analysis was performed. Pituitary and adrenal glands samples were dissected out and fixed with 4% formaldehyde phosphate for 18 hours, dehydrated in alcohol, embedded in paraffin wax, 5µm thick section were cut and stained with hematoxylin and eosin (H&E) for examination.

Statistical Analysis

All the obtained results were analyzed statistically. Using a level of significance of ($p<0.05$), Statistical analysis was carried out by one-way ANOVA followed by Duncan's multi range post hoc test.

Results

Body Weight

Determination of body weight is a major physical indicator of altered body mass. Body weight of all animals was measured regularly. Significant variations were found in the body weight of all groups. Significant ($p<0.05$) rise in the body weight of rats of HS and HF groups was observed in comparison to control group throughout the trial. Significant elevation in the body weight of treatment group

HS+C was also noted in comparison to both control and HS groups. While in HF+C treatment group, significant reduction was found in comparison to control and HF groups.

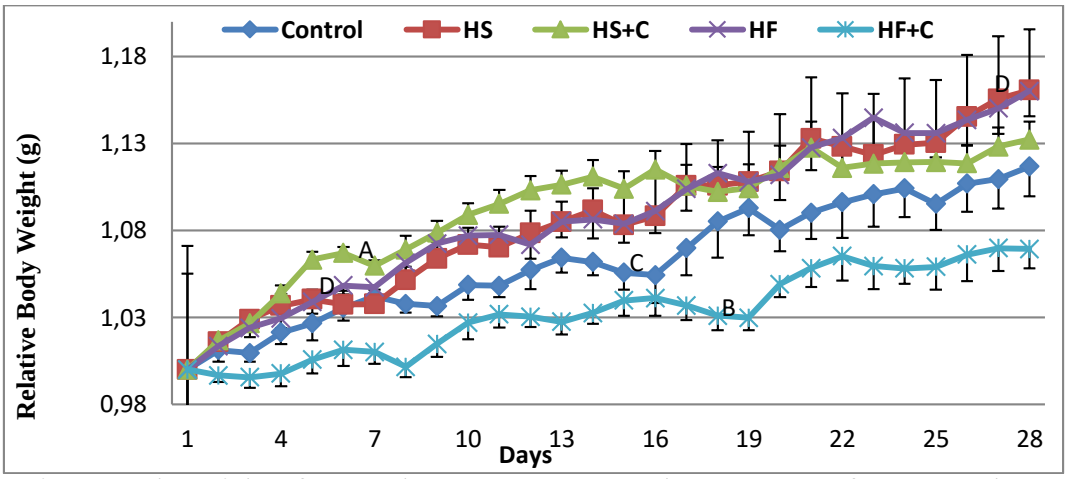


Fig: 1. Body weight of Control, HS, HS+C, HF and HF+C group from 0-28 days

Graphical representation of body weight variations in different experimental groups after arbitrarily adjusting the initial body weight of each group as one.

Glucose Tolerance Test

In order to determine insulin insensitivity oral glucose tolerance test (OGTT) was performed at 28th day of trial after a fasting period of 08 hours. After determining the fasting level of glucose (0 min concentration), a quantity of 5 ml of 10% dextrose solution was given intragastrically to each rat one by one. Blood glucose level was then checked at 60 and 120 minutes respectively. Blood used for said purpose was drawn from tail vein and On Call® Plus glucometer was used to determine the concentration.

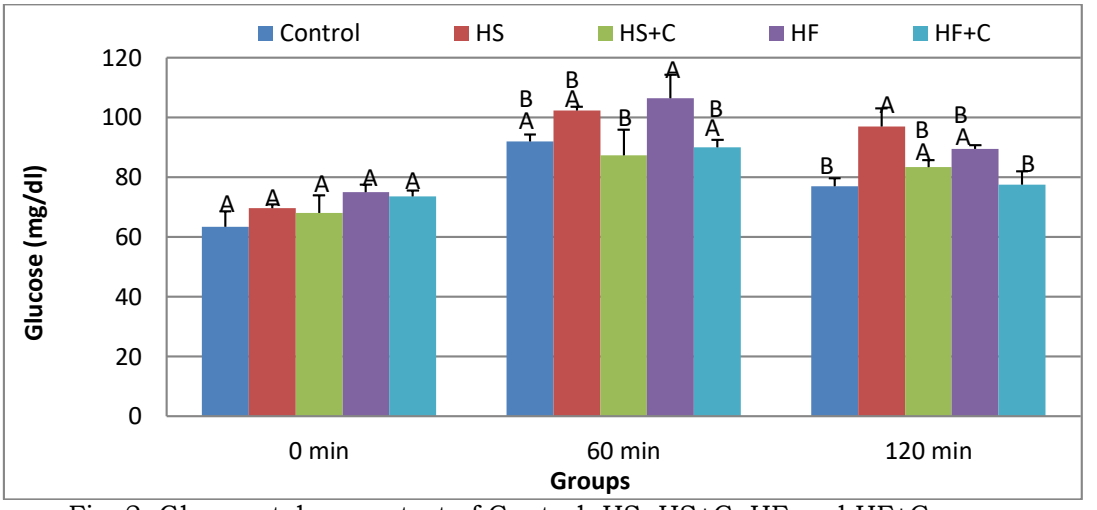


Fig: 2. Glucose tolerance test of Control, HS, HS+C, HF and HF+C group

OGTT of control group showed normal trend as glucose levels returned towards normal level quickly following oral administration of glucose. At 0 min, a non-significant ($p>0.05$) elevation in glucose concentration was noted in HS, HF, HS+C and HF+C groups as compared to control. Highest elevation in glucose level was observed in HF and HS group at 60 min, a significant ($p<0.05$) rise in blood glucose concentration was seen in HF and HS group compared to control after 120 min. The serum glucose level of HS+C and HF+C was significantly lowered compared to HS and HF however not reached at control level.

Hematological Analysis

Concentration of red blood cells (RBC) is an important marker for several pathological conditions. RBC count of animals of all groups was determined. RBC count of HS, HF, was significantly varied against HS+C and HF+C groups ($p>0.05$) and control. While RBC count of treatment groups HS+C and HF+C was significantly increased however level not reached at control value.

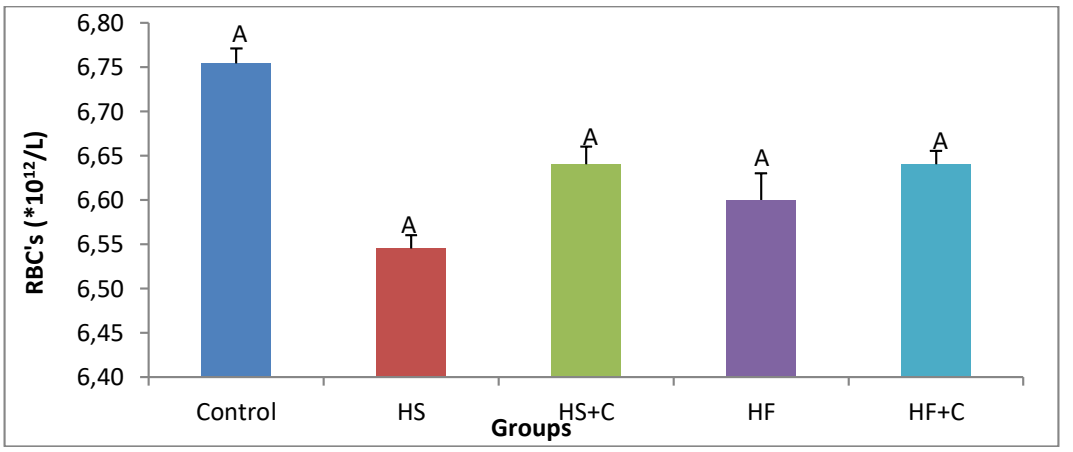


Fig: 3. RBC count of Control, HS, HS+C, HF and HF+C group

A significant reduction in Hb concentration of HS, HF, against HS+C and HF+C groups was found in comparison to control group. Hb concentration of HS group was significantly reduced in comparison to HS+C group. Similarly, Hb concentration of HF group was also found significantly lower in comparison to HF+C group.

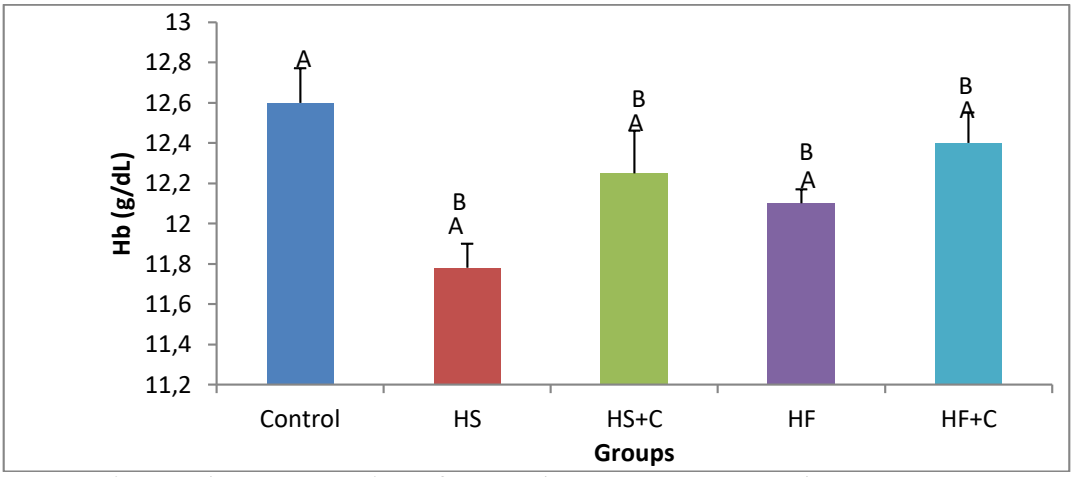


Fig: 4. Hb concentration of Control, HS, HS+C, HF and HF+C group

WBC count is an important marker for various inflammatory diseases. WBC count of HS and HF groups was significantly ($p>0.05$) higher, while WBC count of HS+C and HF+C groups was significantly lower in comparison with HS and HF groups respectively.

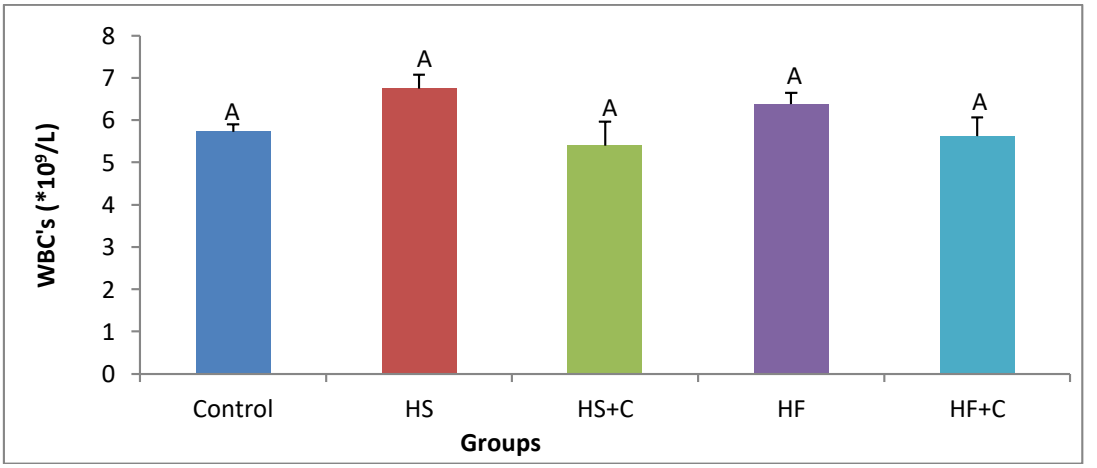


Fig: 5. WBC count of Control, HS, HS+C, HF and HF+C group

Platelet count was elevated non- significantly compared to control in HS and Cinnamon had not exhibited any protective effect. However, the Platelet count in HF was significantly decreased compared to control ($p>0.05$) and was normalized by sub-acute exposure of cinnamon treatment.

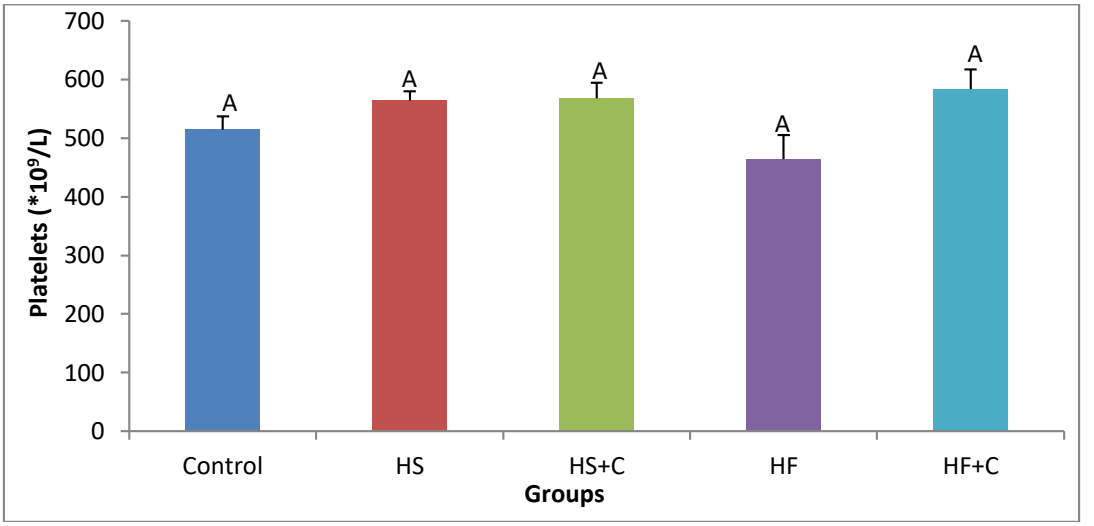


Fig: 6. Platelet count of Control, HS, HS+C, HF and HF+C group

Serum Cortisol Analysis

Serum Cortisol is an important indicator of stress. A significant ($p<0.05$) elevation in the serum Cortisol level was observed in HS and was ameliorated by sub-acute exposure of cinnamon as no significant difference was observed between control and HS+C. Similarly, the serum cortisol level was also increased in HF groups in comparison to control. Serum Cortisol concentration was reduced after sun-acute exposure of cinnamon in HF+C group as non- significant difference was found compared to control.

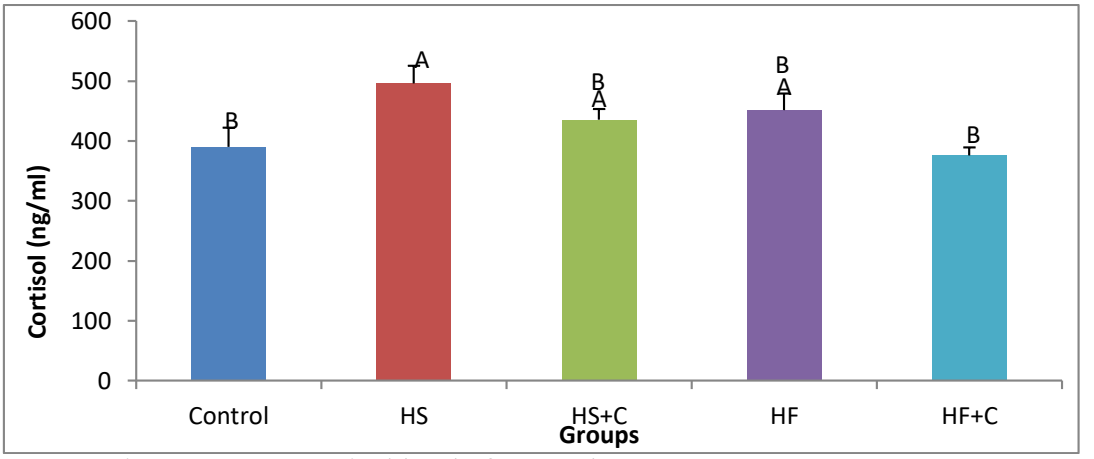


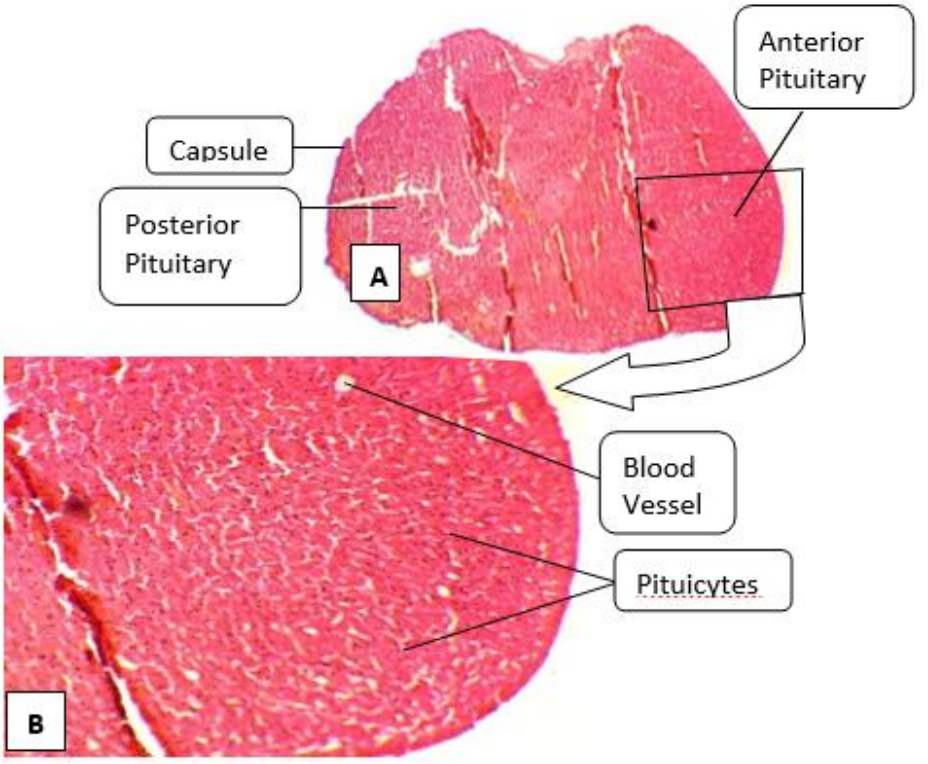
Fig: 6. Serum Cortisol level of Control, HS, HS+C, HF, HF+C group

Histopathological Analysis

Histopathological examination of both pituitary and adrenal gland was performed to evaluate cellular changes upon exposure to high sugar, high fat, Cinnamon with high fat and high sugar. Pituitary gland of control group was normal in

appearance with intact outer capsule and proper cellular arrangement. Blood vessels were normal in appearance and clearly visible (Fig 4.6.1A). However, sections of high sugar and high fat diet groups revealed ruptured capsule and impaired tissue integrity (Fig 4.6.1. B, D). Blood vessels were also irregular in shape and difficult to locate. These finding suggest detrimental effects of continuous consumption of high sugar and high fat diet on pituitary gland. Cinnamon treatment groups receiving cinnamon in addition to high sugar and high fat diet cells were relatively less damaged compared to HS and HF groups, indicating ameliorative effects of cinnamon (Fig. 4.6.1 C, F).

Similarly, adrenal gland of control group was also normal in appearance with clear demarcation of all three cortical regions including zona glomerulosa, zona fasciculate and zona reticularis. Outer capsule was intact with appropriate tissue integrity. Blood vessels were normal in shape and size and were clearly visible (Fig 4.6.2.A). On the other hand, sections of high sugar and high fat diet groups appeared damaged and their capsules were slightly ruptured. It was also difficult to demarcate different cortical regions (Fig 4.6.2. B, D). These results indicate damaging effects of chronic consumption of high sugar and high fat diet in adrenal gland. On the other hand, tissue sections of groups treated with cinnamon demonstrated relatively normal morphology as compared to HS and HF groups demonstrating protective effects of cinnamon (Fig. 4.6.2 C, F).



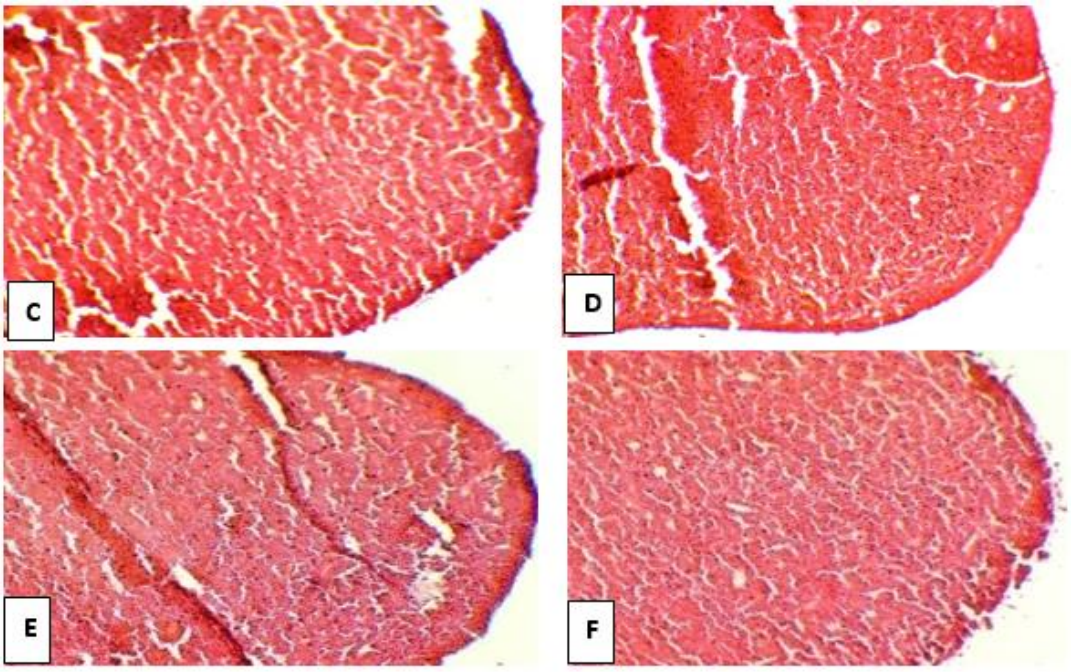
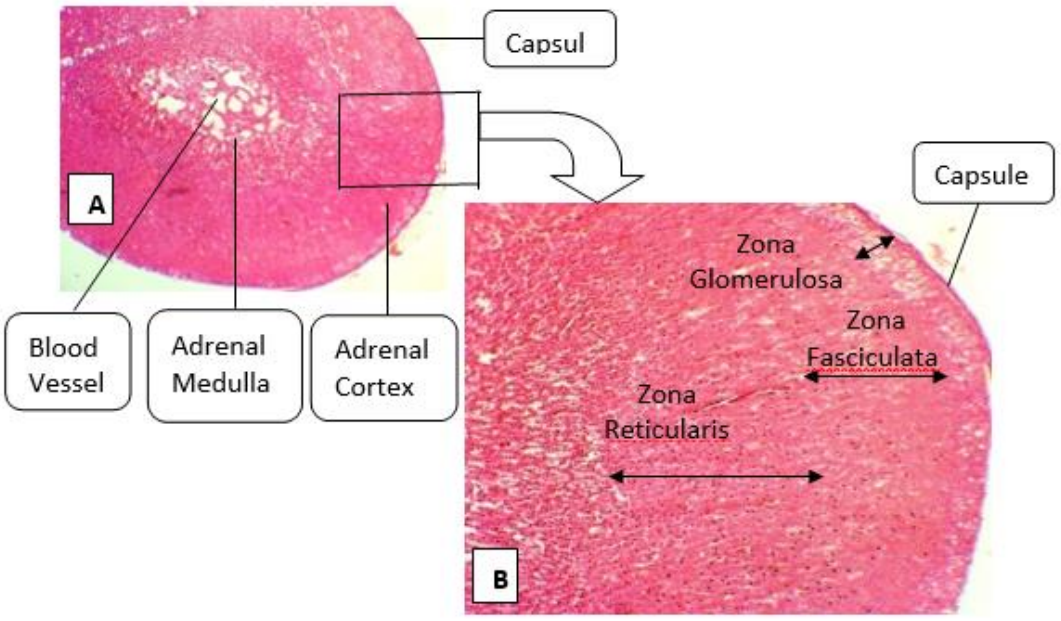


Fig. 4.6.1. Representative photomicrographs of pituitary gland. Pituitary gland of control group at 4X **(A),** Anterior pituitary of control group **(B),** HS group **(C),** HS+C group **(D),** HF group **(E)** and HF+C group **(F)** at 10X.



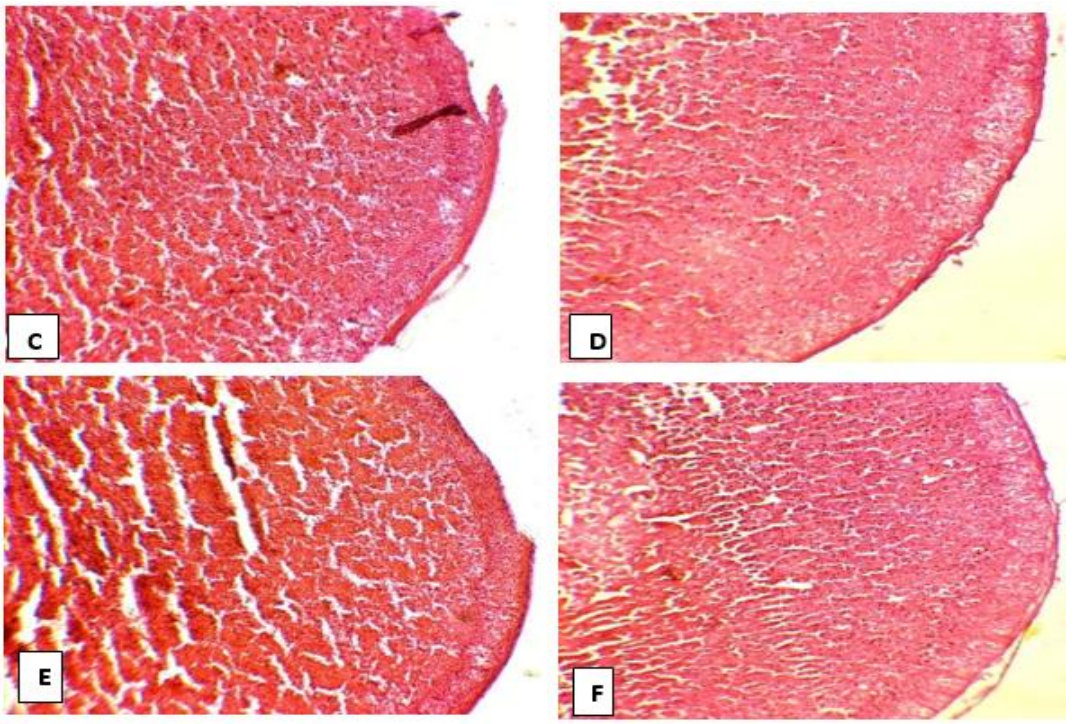


Fig. 4.6.2. Representative photomicrographs of Adrenal gland. Adrenal gland of control group at 4X (**A).** Adrenal cortex of control group **(B**), HS group **(C**), HS+C group **(D),** HF group **(E)** and HF+C group **(F)** at 10X.

Discussion

Obesity has become the most challenging universal health problem (Wronkowitz *et al.,* 2014) being multifaceted, chronic metabolic disorder that badly influences human health. An exponential increase in obesity prevalence has been recorded in the last 3 decades. Rate of childhood obesity has been doubled while rate of adult obesity has been tripled. Obesity has insightful impacts on morbidity, mortality and health care expenditure becoming a public health burden (Hedley, 2004). In addition to a number of other factors, energy dense foods like high fat or high sugar diet have significant contribution in the pathogenesis of obesity. Obesity can lead to a variety of other complications by affecting different body organs including endocrine glands. One of the many complications of obesity is dysregulation of PA axis by altering release of ACTH from anterior pituitary and resultantly cortisol from adrenal cortex. In view of previous literature, current study has focused on obesity associated physiological alterations in PA axis and extent of therapeutic potential of cinnamon towards regulating PA axis. Disproportionate consumption of caloric dense foods results in substandard chronic inflammation due to extension of adipose tissue through adipocyte hyperplasia and hypertrophy. Furthermore, it promotes phenotypic and secretary changes in immune cells of adipose tissue with augmented expression of proinflammatory cytokines (TNFα, interleukine 6 & 8) and diminished expression of adiponectin (Martins *et al.*, 2017).

Cinnamon has been used as preservative and pharmacological agent for long (Rao and Gan, 2014). Cinnamon is full of anti-obesogenic polyphenolic agents that inhibit adipocyte differentiation (Ogasawara *et al.,* 2010), lipogenesis and absorption of fats from small intestine. It also improves glucose tolerance by minimizing insulin resistance (Camacho *et al.,* 2015). Cinnamon has been shown to trigger thermogenic and metabolic reactions in subcutaneous adipocytes in an attempt to fight obesity (Jiang *et al.,* 2017). Cinnamon does not contain many vitamins or minerals, but it is loaded with antioxidants that tend to minimize oxidative stress resulted by hypercortisolism in obesity. Cinnamon has shown beneficial effects in reducing stress responses at a dose of 200 mg/kg (Saxena and Saxena, 2012) administered for a period of two months by lowering blood glucose and cholesterol levels while increasing insulin sensitivity in hyperglycemic individuals (Anderson *et al.,* 2016). Parallel to available information, higher cortisol levels were found in high sugar and high fat diet groups while these levels were significantly lower in Cinnamon treated groups.

Our study has explored the anti-obesity potential of Cinnamon (*Cinnamomum verum)* against high sugar and high fat induced hyper-activity of pituitary-adrenal (PA) axis and obesity in female rats. The high sugar and high fat induced increase in body eight was decline by Cinnamon administration. Hematological analysis revealed a significant decreased concentration of RBCs and hemoglobin and increased WBCs and platelet count following sub-acute exposure of high sugar and high fat changes were attenuated by cinnamon. High sugar and fat associated elevated serum cortisol was normalized after cinnamon exposure. Photomicrograph of pituitary exposed high sugar and high fat diet revealed ruptured capsule and impaired tissue integrity. Blood vessels were also irregular in shape and difficult to locate. These finding suggest detrimental effects of continuous consumption of high sugar and high fat diet on pituitary gland. Cinnamon treatment groups receiving cinnamon in addition to high sugar and high fat diet cells were relatively less damaged compared to HS and HF groups, indicating ameliorative effects of cinnamon against obesity associated dysregulation of PA axis and metabolic impairment.

Photomicrograph of adrenal gland exposed to high sugar and high fat diet appeared damaged and their capsules were slightly ruptured. It was also difficult to demarcate different cortical regions. These results indicate damaging effects of chronic consumption of high sugar and high fat diet in adrenal gland. On the other hand, tissue sections of groups treated with cinnamon demonstrated relatively normal morphology as compared to HS and HF groups demonstrating protective effects of cinnamon.

Consumption of high levels of dietary fat (15g/rat/day in 50g of feed) is a major obesogenic factor (Oliveira *et al.,* 2011) by activating the hypothalamus-pituitary-adrenal axis. Consequently, excess cortisol is produced that is implicated in the development of entire spectrum of the metabolic syndrome, including obesity (Yamauchi *et al.,* 2002). Similarly, consumption of high sugar diet (15g/rat/day in 50g of feed) also stimulates body weight gain. Moreover, it leads to oxidative stress, glucose intolerance as well as dyslipidemia (Moreno-Fernández *et al.,* 2018). Lower weight gain was observed in Cinnamon treated groups indicating beneficial effect.

As a result of chronic use of energy dense foods, fat start to build up at different locations inside the body including adipose tissue, liver and muscles. Excess glycogen also starts to accumulate in hepatocytes (Bawden *et al.,* 2017) leading to glycogen storage disease. This disease gradually affects liver's ability to store iron and consequently progresses towards iron deficiency anemia (Wang *et al.,* 2012; Norazmir, Ayub, and Ummi, 2010; Rojas *et al.,* 2018), as it is evident from lower RBCs count and Hb level observed in high sugar and high fat diet groups. Following Cinnamon administration, RBC count and Hb level were elevated towards normal.

Obesity is strongly connected to persistent inflammation (Gu *et al.,* 2018). High calorie diet induces a quick and transient inflammatory state that stimulates immune cells to resolve the pathology. Chronic intake of such diet leads to persistent inflammation and gradual increase in immune cells (Williams *et al.,* 2014). Adipose tissue in addition to storing energy, acts as an endocrine tissue by releasing a number of different chemical substances having diverse functions. Leptin and adiponectin are the most important and widely studied adipokines. Both are considered as pro-inflammatory substances as they stimulate production of WBCs and increase its count (Dixon and O' Brien, 2006). Higher WBCs count is a risk marker for developing metabolic syndrome (Babio *et al.,* 2013). Thus, as the adipose tissue builds up in the body following intake of high calorie diet, synthesis of adipokines is enhanced that act as stimulus for WBCs formation (Kim and Park, 2008). Similar to previous investigations, higher WBC count was noted in high sugar and high fat diet groups while relatively lower count was noted in Cinnamon treated groups.

Glucose intolerance, induced by chronic consumption of energy dense foods, is considered as an etiological factor for the development of insulin resistance and obesity (Williams *et al.,* 2014). Glucose intolerance associated obesity has been demonstrated by multiple studies on rats fed with high sugar and high fat diets. Significant elevation of blood glucose was noted when measured at 30, 60, 90 and 120 min following oral administration (Kaur and C, 2012). In present investigation, glucose intolerance was observed in both high sugar and high fat diet groups while Cinnamon treated groups exhibited relatively improved tolerance. Glucose concentrations were determined at 0 (fasting), 60 and 120 (postprandial) min following an intragastric administration of 10% dextrose solution.

Conclusion

Current investigation has revealed that both high sugar and high fat diets act as obesogenic factors and lead to obesity with later being more potent. Obesity disrupts the endocrine activity of pituitary and adrenal glands leading to hyper activation of pituitary-adrenal axis. Higher serum cortisol is principal indicator of dysregulation of pituitary-adrenal axis. Hematological analysis of high sugar and high fat groups has revealed reduction in red blood cell count and hemoglobin concentration, while an elevation in white blood cell count. In addition, insulin insensitivity was observed when measured by oral glucose tolerance test. On the other hand, in Cinnamon treated groups, values of all the above mentioned parameters were found normal and closely similar to control group. Thus, it can

be concluded that aqueous Cinnamon extract exhibits protective and ameliorative effects against obesity associated dysregulation of pituitary-adrenal axis.

Declarations

Ethics Approval
The study was conducted in Institute of Physiology and Pharmacology, University of Agriculture. All methods were performed in accordance with relevant guidelines and regulations of "Local Ethics Committee for Animal Experiments".

Consent for Publication
Not Applicable

Availability of Data and Materials
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests
Authors don't have any competing interest.

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Authors' Contributions
MA and JAK designed the study. MA conducted the experiments with the help of other authors. JAK provided reagents and guidance. JAK analyzed data and MA wrote the manuscript.

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