



Research Article

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## The Effects of *Withania somnifera* on Blood sugar, Serum Insulin, Lipid Profile and Liver Enzymes in Fructose Drinking Water Male Rats

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### ABSTRACT

Insulin resistance is a metabolic disorder which alters the liver inducing *de novo* lipogenesis and gluconeogenesis. The aim of the present study is to evaluate the chronic administration of *Withania somnifera* Root powder (WS) on blood sugar, lipid profile, and liver enzymes in fructose drinking water (FDW) rats. At the beginning, 48 Wistar-Albino male rats, weighting  $200 \pm 20$  g were divided into control (C) and (FDW) group (n=24): half of C and FDW groups received 62.5mg/g diet WS root powder (CWS & FWS groups) during trial period (n=12). Group C did not receive any agent but group FDW received fructose-enriched water (10%, w/v) during trial period. At the end of the experiment, animals were anesthetized by diethyl ether, sacrificed and blood samples were collected from cervical vessels. Serum insulin, blood glucose, lipid profile and liver enzymes were measured. Obtained data were analyzed by SPSS Software Ver. 18; using ANOVA and tukey test. Results were expressed as mean  $\pm$  SD. Statistical differences were considered significant at  $p < 0.05$ . The results of the present study showed that Blood glucose, serum insulin, insulin resistance index, triglyceride, and AST and ALT in group FDW were significantly higher than groups C and CWS but this values in group FWS decreased significantly compared to FDW group ( $p < 0.05$ ). Our results indicated that chronic administration of *witania somnifera* root powder in diet improved blood glucose, serum insulin, insulin resistance index and liver enzymes in FDW rats.

**Keywords:** *Withania somnifera*, ALT, AST, Insulin resistance, blood sugar.

### INTRODUCTION

Metabolic syndrome (MS) is a bunch of pathophysiological disorders correlated with abdominal obesity, insulin resistance, high blood pressure, and dyslipidemia (1). Consumption of high-fructose corn syrup (HFCS) has a sequential relation to the epidemic of obesity, and the overconsumption of HFCS in calorically sweetened material may play an important function in the epidemic of obesity (2). Metabolic syndrome can be grounded by alteration of diet by the way of utilization of high carbohydrate such as fructose and high fat diet (3). Chronic fructose feeding leads to glucose intolerance, hyper-insulinemia, and loss of normal insulin sensitivity which was associated with insulin resistance index (4). Insulin resistance index (IRI) is a quality and it is defined as a position in metabolic syndrome, and diabetic (type 2) patients in which target cells responsiveness decreased to normal circulating levels of insulin (5). Experimental study has shown that fructose-rich diet shows signs of insulin resistance index and causes hypertension similar to metabolic syndrome and human type II in laboratory animal model (6). Fructose

diet-induced hyperlipidemia alters insulin sensitivity which is correlated with decrease in tissues insulin action in animal model (7). High levels of fructose in drinking water results in metabolic abnormalities and insulin resistance, and vascular disorders(8).Increased fructose use is associated with increasing incidence of different metabolic disorders, which were interconnected to oxidative stress ,lipid peroxidation ,and protein damage in the liver (9 ,10). Medicinal plants are vital foundations of potentially positive new compounds for the development of effective therapy and have the advantages of better affordability and acceptability, better compatibility with the human body, and minimal side effects (11 ).*Withania somnifera* (W.S) is a herbal plant, in the Solanaceae family, widely used in traditional medicine for a number of disorders. W.S is growing in Africa, Mediterranean, and India (12, 13). Oral administration of the W. S plant extract caused a significant benefit in infected Guinea pig (14).W.S roots extract is a new approach for the treatment of alcohol misuse, diabetes-induced testicular dysfunctions and did not show any toxicity effects in rats (15-17).

The aim of the present study is to assess the chronic administration of W.S root powder on blood glucose, serum insulin, insulin resistance index, lipid profile and liver enzymes in FDW rats.

### EXPERIMENTAL SECTION

At the beginning of the test ,48 healthy male Albino- Wistar rats, weighing  $200\pm 20$  gr, were selected from Zahedan University of Medical Sciences animal house, and maintained under controlled condition of artificial (Timer Model: SUL180a, AC220V. China, 6 Am to 6 Pm), illumination (a: 12-hr light: dark cycle), temperature  $21-25^{\circ}\text{C}$  and humidity of 45-65% and the air was suitably recycled. Animals had free access to food and water intake throughout the experimental period. After five days of research, animals were weighed by EK-b10 Japan digital balance (first weight). Based on their weight, animals were randomly divided into 4 groups (n=12) as follows: C, CWS, FDW and FWS groups and were kept in the cages (one rat in cages).

Control group (C) did not receive any agents during experimentation period.

Control group CWS: received water tap and rodent's diet which contain 62.5mg/gram diet *Withania somnifera* root powder on daily basis for 8 weeks (18).

Group FDW: intakes fructose-enriched water (10%, w/v) and ordinary rodent's diets during experimental period (19).

Group FWS: received fructose-enriched water (10%, w/v), and rodent's diet containing 62.5mg/gram diet of *Withania somnifera* root powder (18, 19).

#### Preparation of the extracts

*Withania somnifera* was collected from limited area around Shiraz, Iran in August 2013 and were recognized by the Centre of Taxonomy of biology in Faculty of Science, Sistan and Baluchestan University, Zahedan, Iran. *Withania somnifera* root (WSR) was broken up with awareness, shade-dried in a room temperature and then altered in to powder. Basal diet was diverse with 62.5mg/g WSR powder and water in order to produce new rodent's diet pallets (19). Food and water intake was measured at the end of first 20 days, second 20 days ,and third 20 days (F1, F2, F3, Wi1, Wi2 and Wi3).

At the end of experiment (8 weeks) and after overnight fasting (12-14 hours), animals were weighed again by the same digital balance (final weight) and then were deeply anesthetized by diethyl ether (Merck Germany), sacrificed, and blood samples were immediately collected under standard condition from cervical vessels.

All blood samples were collected in ordinary vials and centrifuged at 3000rpm for 10 minutes in classifies to separate serum. Serum was removed (BH-1200 type Iran) and stored at  $-70^{\circ}\text{C}$  for more analyses. Blood glucose was measured by glucose oxidase method. Serum insulin was measured by sensitive rat kit (DRG, instruments, GMBH, Germany,), using double antibody Enzyme-linked immunosorbent assay (ELISA). Homa -IR index was obtained from the following formula:  $\text{Homa -IR} = \text{Fasting insulin } (\mu\text{L}) \times \text{Fasting plasma glucose (m mol/L)}/22.5(20)$ .

Serum AST and ALT, triglyceride (TG) and total cholesterol (TC) were measured using the usual methods modified for RA 1000 Analyzer (Technicon, USA) with Pars Azmon kit Iran. Serum high density lipoprotein (HDL) was determined by precipitation of non-HDL lipoprotein with dextran/MgSO<sub>4</sub>, followed by enzymatic cholesterol assay. Low density lipoprotein (LDL) was estimated according to Frirdewald formulas (21).

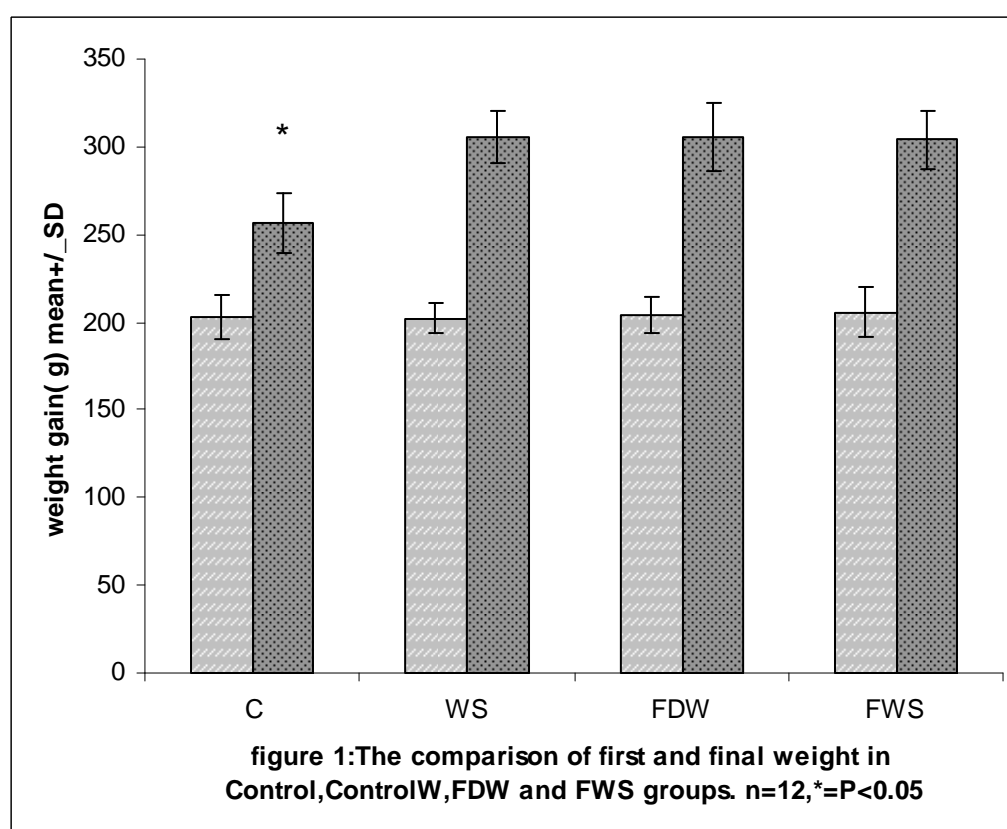
**Statistical analysis:** The normal allocation of data was standard through Kolmogorov-Smirnov test, and then all data were assessed by SPSS software v. 18; via ANOVA and Tukey tests. The results were expressed as mean  $\pm$  SD. Statistical differentiations were considered significant at  $p < 0.05$ .

The present study followed the majors and agreement of the Iranian Council on Animals, and obtained institutional ethical sustain from the committee for Animal Research, Zahedan University of Medical Sciences (6103).

## RESULTS

Results obtained from the present study showed that insulin resistance indices (IRI), in group FDW was significantly higher than that of C, CWS groups but this value in FWS group decreased significantly compared to group FDW (table 1).

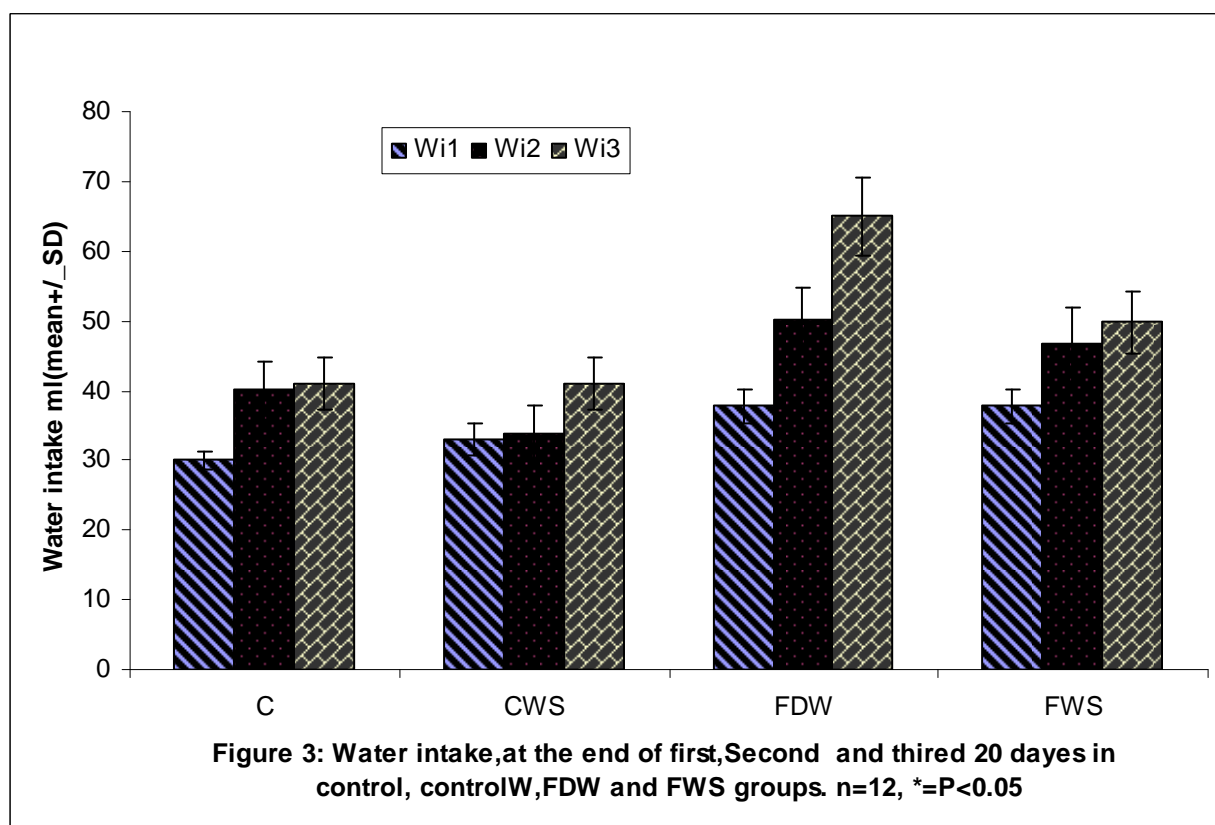
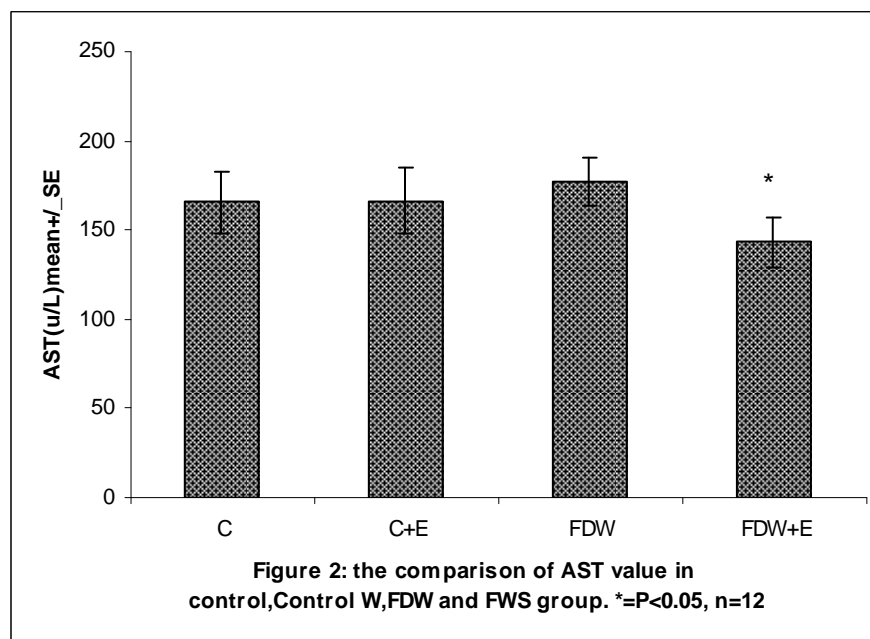
Moreover, cholesterol and triglyceride values in group FWS decreased significantly compared to FDW group, but these values in other groups did not show any differences (table 2). The comparison of the first weight in all groups did not show any differences but the final weight in CWS, FDW, and FWS groups were significantly higher than group C (figure 1).



**Table 1: The effects of *Withania somnifera* root powder on blood sugar, serum insulin, insulin resistance index in Fructose Drinking Water rats**

Parameters↓	groups				P value
	C	CWS	FDW	FWS	
IRI	0.06 $\pm$ 0.04	0.06 $\pm$ 0.02	0.32 $\pm$ 0.13	0.15 $\pm$ 0.11 *	P<0.001
Insulin( $\mu$ L)	0.24 $\pm$ 0.14	0.22 $\pm$ 0.08	0.97 $\pm$ 0.37	0.54 $\pm$ 0.39 *	P<0.001
FBS(mg/dl)	112 $\pm$ 7.32	110 $\pm$ 6.95	133 $\pm$ 9.3	114 $\pm$ 5.68*	P<0.001

Based on ANOVA and Tukey test, IRI, insulin and FBS values in group FDW were significantly higher than that of C, CWS, but these values in group FWS decreased significantly compared to FDW group. The results were expressed Mean  $\pm$  SD, n = 12 in each group. \*P < 0.05



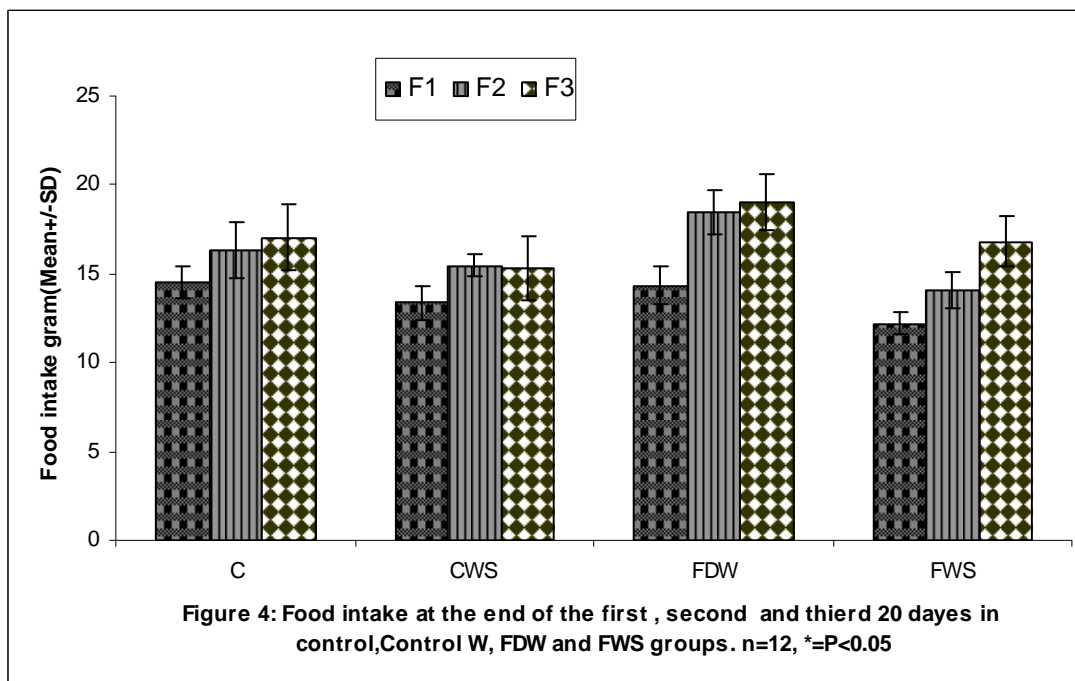
Furthermore, our findings revealed that AST value in group FWS decreased significantly compared to FDW group (Figure 2).

In addition, our results showed that WI3 and F2 in group FWS decreased significantly compared to group FF (figure 3 and 4).

**Table 2: The effects of *Withania somnifera* root powder on Cholesterol, Triglyceride, HDL and LDL in FDW rats**

Parameters↓	groups				P value
	C	CWS	FDW	FWS	
Cholesterol mg/ml	72.83±17.18	71.16±12.37	85.83±4.46	74.75±6.56 *	P<0.012
Triglyceride mg/ml	49.33±9.7	41.50±7.94	58.91±15.61	46.50±5.66 *	P<0.002
HDL mg/ml	34.75±5.7	35.58±6.47	35.1±4.88	40.33±4.55	P<0.063
LDL mg/dl	28.23±12.08	27.28±7.11	23.63±5.6	28.3±5.03	P=0.46

Based on ANOVA and Tukey test, Cholesterol and Triglyceride in group FDW were significantly higher than C and CWS groups, but these values in group FWS decreased significantly compared to FDW group. In addition, HDL values in group FWS decreased compared to other groups but did not show statistical significance. LDL values among all groups did not show any differences. The results were expressed as Mean ± SD, n = 12 in each group. \*P < 0.05



## DISCUSSION

Our findings in the present study show that FDW consumption (10% w/v) for 8 weeks induced insulin resistance index (IRI), serum insulin, Fasting Blood Sugar in male rats but these values in group FWS decreased significantly compared to group FDW.

Moreover, our results in the present study indicated that the weight gain increased in CWS, FDW, and FWS groups compared to group C. The results obtained from the present study showed that cholesterol and triglyceride values in group FDW increased significantly compared to group C and CWS but consumption of *Withania somnifera* cause decreases of these values in group FWS.

Fructose is a monosaccharide which is consumed in considerable number of Western and modern diets and its utilization has increased in the past few decades. Fructose consumption has been joined together with a produce in obesity and metabolic disorders (22). Fructose is a carbohydrate which is found in fruits and it is used as sweetener in foods and drinks and its utilization is related to incidence of abdominal obesity, insulin resistance, metabolic syndrome, and type II diabetes mellitus (23). Moreover, insulin and leptin are two important hormones which act on food intake and body-weight gain regulation in all vertebrates and humans (24). Insulin resistance and hyperinsulinemia are common results in patients with MS and type II diabetes, and essential hypertension, which are high risk factors in cardiovascular diseases and development of coronary artery disease (25). Our findings in the present study show that FDW consumption induced insulin resistance index (IRI), hyperinsulinemia, hyperglycemia in male rats. These results were supported by previous studies in that fructose drinking water enriches promotion of insulin resistance and hyperinsulinemia in experimental animal model (23). Moreover, the results in present study showed that cholesterol and triglyceride values in group FDW increased significantly in comparison with group C and CWS but chronic consumption of *Withania somnifera* decreases these values in group FWS and was supported

by Hurjui DM *et al* (25). Repeated admin of aqueous extract of *Withania somnifera* coagulants (AWC) has shown a significantly anti-hyperlipidemic and hypoglycemic activity in diabetic rats which was induced by streptozotocine rats (26). *Withania somnifera* root extract has shown antihyperlipidemic activity in high-cholesterol diet-induced in albino rats and confirmed our results (27). Postic C *et al* in 2008 reported that consumption of fructose causes elevated serum triglyceride and fatty liver by stimulating de novo (31). The previous experimental study indicated that *Withania coagulans* administration in diabetic rats which was induced by Streptozotocin reduces the occurrence of oxidative stress and inflammation and improves hyperglycemia (28). This report supports our findings which indicated that diet containing *Withania somnifera* root powder improved hyperglycemia in FDW. The previous experiment revealed that WSR and leave extracts contain the phenolic compounds and flavonoids improving liver injury in diabetic rat after 8-week administration by restore the, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and reduced glutathione (GSH) in rat liver (29). Anwar MF *et al* reported that administration of WS could significantly improve AST and ALT values *in rat* liver which were damaged by silver nanoparticle (30).

Additionally, our findings revealed that AST value in group FDW was significantly higher than group C and CWS but this value in group FWS decreased significantly compared to group FDW. Our finding revealed that food intake at the end of second 20 days and water intake at the end of third 20 days in group FWD increased noticeably compared to those of group C and CWS but this value in group FWS decreased significantly compared to group FDW. This section of our results was in disagreement with that of Rivera-Ramírez F *et al* 2012 (33) who reported that food intake in mice fructose feed decreased compared to control group. These differences may be due to differences of animal used in these experiments and the experimental period. On the other hands, these results are in agreements with Mamikutty N *et al* findings (34).

Our findings showed that the weight gain in CWS, FDW and FWS groups increased significantly compared to C group. Al-Rasheed N in 2014 reported that fructose causes increased weight gain in male rats by increasing adipose tissue hyperplasticity and hypertrophy (32). Mirakzahi MT *et al* in 2013 reported that WS supplementation improved calcium retention, bone calcifications and increase bone performance effects (35) and supported this section of our findings.

### CONCLUSION

Our finding in the current study showed that administration of *Withania somnifera* root in rodent diet has shown anti-hyperglycemic, anti-lipidemic effects and causes improved liver damages in FDW rats. The exact mechanism needs further studies

### Acknowledgements

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### Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

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