Journal of Pharmacology Research
ISSN: 0976-7134 & E-ISSN: 0976-7142, Volume 3, Issue 1, 2013, pp.-54-58.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000279

GINSENG (G115) IMPROVES METABOLIC SYNDROME AND SOME ANTIOXIDANT ENZYMES IN ALBINO RATS

KAMAL S.M.*

Pharmacology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
*Corresponding Author: Email- saharkamal2003@hotmail.com

Received: August 02, 2013; Accepted: August 13, 2013

Abstract- Obesity, dyslipidemia, hypertension, and insulin resistance are elements of metabolic syndrome (MS). The present study investigated the effect of ginseng extract (G115) in protection against the development of high fructose-induced MS in rats and its possible antioxidant role in this model. Albino rats were divided into 6 groups: normal control (group 1: received normal diet); high fructose fed (HF) (group 2: received 20% fructose plus saline as untreated MS model; Ginseng extract (G115)-treated (received HF at a dose of 40 mg/kg/day for 6 weeks). Systolic blood pressure (SBP), visceral fat index (visceral fat weight /body weight ratio), insulin resistance, activities of glutathione peroxidase & catalase enzymes & superoxide dismutase enzyme in red blood cells (RBCs) lysates and glutathione replenishing abilities of hepatic cells of tested groups were measured. The results showed that G115 provided a protection against the development of high fructose-induced MS. It significantly reduced SBP to levels comparable to control group on normal diet. It also significantly modified visceral fat index, insulin resistance. Additionally, it increased the activities of tested antioxidant enzymes. These results indicated that high fructose-induced MS could possibly be regulated by ginseng extract (G115) with its ability to lower high blood pressure with a significant antioxidant activity.

Keywords- ginseng extract (G115), metabolic syndrome, SBP, serum lipids, antioxidant activity

Citation: Kamal S.M. (2013) Ginseng (G115) Improves Metabolic Syndrome and Some Antioxidant Enzymes in Albino Rats. Journal of Pharmacology Research, ISSN: 0976-7134 & E-ISSN: 0976-7142, Volume 3, Issue 1, pp.-54-58.

Introduction

Metabolic syndrome (MS) is a life -threatening syndrome involves a number of co-abnormalities notably obesity, dyslipidemia, hypertension, and insulin resistance [1]. Diabetes mellitus, acute myocardial infarction and inflammatory joint diseases are associated with MS which indicate the use of drugs suitable for the control of each disease [2].

Panax ginseng (G115) is known by its ability to improve general body conditions and to provide a proper therapeutic effects in treatment of many diseases as a traditional chinese medicine.

Experimental studies on rodents revealed the therapeutic benefits of Korean Red Ginseng (KRG) in treatment of cardiovascular diseases and some metabolic disorders [3-5]

However, little knowledge could be collected about the therapeutic effects of different types of ginseng either G115 or KRG on metabolic syndrome [6,7].

The traditional Chinese medicine (TCM) shows a beneficial role in management of MS. However,no obvious measurements related to diseases included in MS have been recorded in many studies. Panax Ginseng extract is one of three Chinese herbs that were under focus to explain its possible therapeutic potentials in diabetes mellitus, obesity and oxidative stress [8,9]. Further evaluation of its possible therapeutic mechanism (s) in MS will be of great value to build up a proper hypothesis about its therapeutic benefit as an alternative and complementary medicine in such syndrome.

The present study investigates the possible therapeutic effect (s) of ginseng extract (G115) on systolic blood pressure, insulin resistance, some anti-oxidant enzymes and glutathione replenishing ability of hepatic cells in an experimental model of metabolic syndrome.

Materials and Methods

Animals

Albino Wister rats (150-200 g) were purchased from the animal house (Bilharzial Center of Research, Ain Shams University, Cairo, Egypt), housed in controlled environmental conditions. They were left for one week for acclimatization. They were housed in cages with a natural light-dark cycle and fed on a standard pellet diet and water ad libitum.

Chemicals

Ginseng extract (G115) was supplied as yellow powder [Ginsana Products Lugano, SA, (Switzerland)]. *N-(l*-naphthyl)ethylenediamine dihydrochloride (ICN Biomedicals Inc., Egypt) and diethyl maleate (Merck Biochemicals, Egypt). All other chemicals were purchased from Sigma chemicals co.

Experimental Design

Induction of Metabolic Syndrome [MS]

Metabolic syndrome was induced by dissolving 10 gram fructose in 100 ml drinking water, besides a 10% fructose in diet (i.e. adding 10 gram fructose per 100 gram normal diet). The total ingested fructose was accordingly 20% for 6 weeks. This method was modified from Faure, et al [10].

Animal Grouping

Albino rats were divided into 6 groups (N= 8 rats in each group).

They were classified as follows:

Group 1: a normal control which received normal diet.

Group 2: high fructose group (HF) received 20% fructose (10% with diet (weight/weight) and 10% with water (weight/volume)) and injected with saline to serve as control MS group

Group 3: ginseng extract (G115)-treated HF group (HF+ G115) received HF plus G115 (40 mg/kg/day, ip) (the dose of G115 was chosen according to Rhee, et al [11].

Administration of G115 began simultaneously with fructose feeding and continued allover the duration of the study which is 6 weeks.

At the end of the experimental period, the animals were weighted, anesthetized with ether. Blood samples were collected from the abdominal aorta as follows: the rat was fixed on a wooden plate and the abdominal cavity was opened, then the abdominal aorta was explored at its bifurcation after gentle traction of the viscera using soft tissue. Blood was centrifuged at 5000 rpm for 10 minutes for serum separation, and kept at -80°C until further measurements.

Measurements

Assessment of systolic blood pressure (SBP) changes

SBP was measured by a tail-cuff sphygmomanometer (UR-5000, Ueda Co, Ltd, Japan). SBP measurements were conducted in all groups over a period of 8 hrs. The SBP readings were taken three times at 30 minutes intervals to reduce variability [12].

Visceral Fat Measurements

Visceral fat (adipose tissue surrounding the abdominal and pelvic organs) was dissected and weighed. Visceral fat index was calculated according to the following equation: (visceral fat weight (g) /g body weight) x 100 [13].

Biochemical Measurements

Fasting blood glucose and serum levels of fasting insulin using commercially available kits, anti-oxidative stress enzymes (glutathione peroxidase, catalase, superoxide dismutase (SOD) were measured as follows:

Insulin Resistance

Fasting blood glucose was measured using blood glucose measurement kit (Boerhinger Mannheim, Mannheim, Germany). Serum insulin was measured by enzyme-linked immunosorbent assay (ELISA) Kit (SPI-BIO, France) according to the kits' instructions. Insulin resistance (IR) was calculated using Homeostasis Model Assessment-insulin resistance (HOMA-IR) calculator program [14].

Determination of Glutathione Peroxidase [GPx] Enzyme Activity

Glutathione peroxidase (GPx) activity in the liver homogenates was measured by the method described by Rotruck, et al [15]. The activity was expressed based on inhibition of GSH.

Determination of Catalase Enzyme Activity

Catalase (CAT) activity in the liver homogenates was assayed colorimetrically as described by Sinha, et al [16] using dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed 1:3 (v:v). Spectrophotometric readings were recorded at 620 nm and the amount of hydrogen peroxide hydrolyzed was calculated for the catalase activity

Determination of SOD Enzyme Level in Erythrocyte Lysates

At the end of the 6th week of the study, blood samples were collected from rats of all groups for measurement of SOD levels in lysates that were prepared from red blood cells (RBCs) of tested rats, using spectrophotometric assay kit as described by Sato, et al [17] and results were expressed in IU/mL.

Glutathione Repletion Experiments

Diethyl maleate (DEM.), a carbonyl compound that rapidly depletes cytosolic glutathione was used to compare glutathione-replenishing abilities of hepatic cells of all tested groups. Briefly, hepatocyte monolayers cut by microtome were exposed to 500 uM DEM for 30 min to achieve glutathione depletion. The media containing DEM were then removed and the cells were washed with Hanks-buffered saline (HBS) before the media were replaced with DEM-free RPMI-1640 media. After 1, 2 and 4 hrs., the plates were washed 3 times after removal of culture media. Wash was done using HBS before 1 ml of trichloroacetic acid (6.5%) was added to precipitate cellular proteins. The cellular matter was then scraped from the plates and transferred to Eppendorff's tubes. After centrifugation, the acidic supernatant was analyzed for glutathione using a procedure based on the method of Saville [18]. The remaining pellets were dissolved in 0.5 ml of 0.5 M sodium hydroxide, and incubated at 40°C for 1 h; protein was then determined using the Bradford method [19]. Glutathione levels were expressed as µmol/mg protein.

Determination of intracellular reduced gutathione levels. Glutathione concentrations were determined by the method of Saville [18]. One milliliter of 6.5% trichloroacetic acid in 0.5 mM EDTA was added to each dish, and then the cells were scraped with a rubber policeman, collected into 1.5-ml microcentrifuge tubes, and centrifuged at 13,000 rpm (Beckman microcentrifuge) for 5 min. The supernatants were transferred to new tubes, the protein pellets were dissolved in 0.5 ml of 0.1 M NaOH each, and the fractions were frozen for al least 24 h before performance of the assay. The thiol determination was carried out with freshly prepared reagents on supernatant fractions in 96-well microplates. Fifty microliters of thawed supernatant was mixed with 50 ul of 10 mM sodium nitrite solution prepared in 0.96% sulfuric acid, and the mixture was incubated for 5 min at room temperature. Ten microliters of 0.5% ammonium sulfamate was added, and the mixture was incubated for 5 min. Then 100 µL of a solution consisting of 1 part 0.5% mercuric chloride and 4 parts 3.4% sulfanilamide in 0.4 M hydrochloric acid was added, and the mixture was incubated for 5 min. The color reaction was developed after a 5-min incubation with 40 ul of substrate solution containing 0.2% (*N-(l*--naphthyl) elhylenediamide dihydrochloride in distilled water. The absorbance at 540 nm was read in a spectrophotometer; the blank solution consisted of 50 µL of 6.5% trichloroacetic acid processed as described for the supernatant fractions. The standard curve was generated from triplicate samples of serial dilutions of reduced glutathione, and the results were expressed in nmoles of glutathione per milligram of protein.

Data Analysis

Results are expressed as mean ± SD [Standard Deviation]. Statistical analysis was performed by analysis of variance followed by Tukey's *post hoc* using GraphPad Prism version 3.00 for Windows 97 (Graph Pad Software, San Diego, CA, USA). Differences with $p < 0.05$ were considered to be statistically significant.

Results

[Fig-1] shows Effect of ginseng extract (G115) on systolic blood pressure (SBP) of tested groups.

The increase in SBP in albino rats with high fructose (HF) diet (group 2) was significantly (**$p < 0.05$) lowered by G115 in group 3. The mean ± SD of SBP for each group remained constant all over the 8 hrs. period of measurement of SBP.

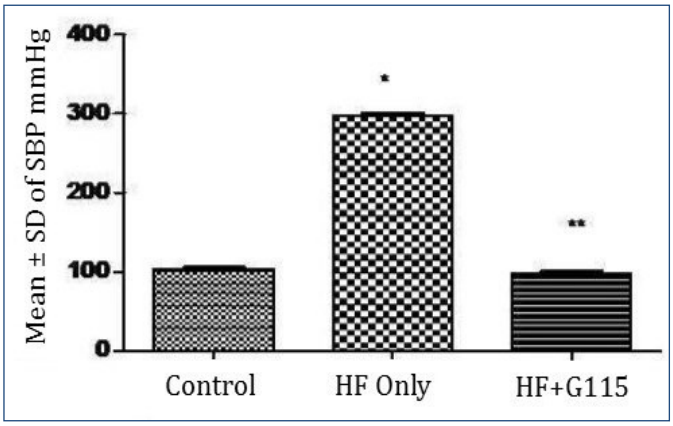

Fig. 1- Effect of ginseng extract (G115) on systolic blood pressure (SBP) of tested groups.

**significant ($p<0.05$) increase in SBP in high fructose (HF) fed-diet group 2 compared to its significant (**) reduction in HF fed-diet + G115 -treated group 3*

[Table-1] shows values of visceral fat weight / body weight ratio (VW/WW ratio), Fasting blood Glucose (mg/dl), Fasting serum insulin (µIU/ml), homeostasis model for assessment of insulin resistance (HOMA-IR) in different tested groups. All results were represented as the mean ± SD. (n= 8 rats/group)

Table 1- Effect of ginseng extract (G115) on some measurements related to metabolic disorders in fructose-induced metabolic syndrome

	Group 1 Control	Group 2 HF fed-diet	Group 3 HF fed-diet +G115
VW/WW ratio	0.31± 0.3	2.2± 0.3*	1.03± 0.23**
Fasting blood Glucose (mg/dL)	94.6 ± 5.98	306± 3.37*	227 ± 5.25**
Fasting serum insulin (µIU/ml)	12.0 ± 1.63	79.0± 5.66 *	50.9 ± 1.87**
HOMA-IR	2.8± 0.3	59.69± 0.05*	28.53± 0.02**
Serum total cholesterol (mg/dL)	90.5±4.3	255.6±23.4*	105.9±12.5**

VW = visceral fat weight.

**p<0.05 = significant increase compared to the control group 1*

***p<0.05= Significant decrease compared to HF untreated diet group 2*

[Table-2] shows a significant decrease in the activities of the measured anti-oxidant enzymes was noted in untreated model rats. G115 -treated group 3 showed that their activities were significantly increased compared to model untreated group 2 as follows:

Table 2- Mean ± SD changes in activities of liver Cat, Gpx enzymes in tested groups of albino rats expressed As Unit/mg tissue protein of liver homogenates.

Group	Catalase Unit[a]/mg tissue protein	Glutathione peroxidase Unit[b]/mg tissue protein
Control group 1	66.32± 2.2	15.23± 2.5
HF-fed non-treated group 2	1.23± 0.32*	0.22± 0.02*
HF-fed treated with ginseng extract group 3	53.25± 3.4**	11.33± 0.79**

[a] = Moles of hydrogen peroxide consumed per minute.

[b] = µg of glutathione consumed per minute.

**p<0.05, significant reduction in activity of both enzymes in untreated model group 2 versus Group 1.*

***p, 0.05, significant increase in activity of both enzymes by G115 versus in untreated model group 2.*

A significant decrease in the activities of these enzymes was noted in HF-fed non-treated rats. G115-treated group 3 showed that their activities were significantly increased compared to model untreated group 2.

This suggests that ginseng extract (G115) could possess an antioxidant activity in case of HF-fed albino rats.

[Fig-2] shows Measurement of SOD enzyme levels (IU/mL) in erythrocyte lysates of all tested albino rats.

Effect of 6-weeks administration of tested drug on levels of superoxide dismutase [SOD] enzyme in IU/mL in erythrocyte lysates of the tested albino rats. Results are expressed as mean ± SD (n = 8 rats/group).

Significant (*$p<0.05$) increase in SOD levels was obtained with Group 3 (treated with G115) when compared to Group 2 (model untreated).

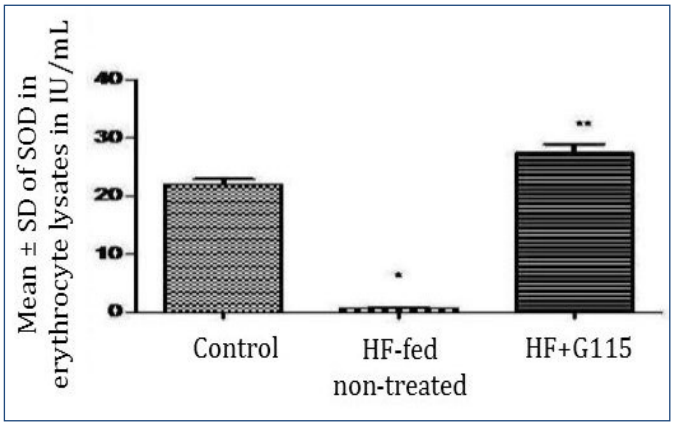

Fig. 2- Measurement of SOD enzyme levels (IU/mL) in erythrocyte lysates of all tested albino rats

**p<0.05= significant decrease in SOD enzyme levels in group 2 compared to the control non-treated albino rats group 1*

***p<0.05 = significant increase in SOD enzyme levels in G115-treated group 3 compared to the HF fed -non treated albino rats group 2*

[Table-3] shows Changes in glutathione levels expressed as nmol/mg protein before and 4 hrs. after exposure to 0.5 mM diethyl maleate (DEM).

Data were obtained from hepatic cells isolated from either control, HF-fed diet, HF diet+G115 treated group as indicated in the [Table-

3]. There is significant (*p<0.05) reduction in glutathione levels in control and HF-fed rats. However, treatment with of HF-fed albino rats treated with G115 for 6 weeks significantly (**p< 0.05) seems to provide protection against acute depletion of glutathione in comparison to groups 1 & 2 suggesting enhanced glulathione replenishing ability upon treatment with ginseng extract (G115) in hepatic cells of HF-fed albino rats.

Table 3- Effect of treatment with ginseng extract (G115) on Glutathione repletion ability of hepatic cells expressed nmoles of glutathione per milligram of protein

Groups	Change in glutathione level		
	Before exposure to DEM	4 hrs. After exposure to DEM	% decrease in glutathione level
Control (group 1)	46.5 ±3.2	6.2 ±2.1*	-86.67%
HF- untreated group 2	47.2 ±2.9	5.2 ±0.7 *	-88.98%
HF + G115 group 3	51.3 ±3.1	42.5 ±2.1**	-17.15%

Results are expressed as mean ± SD
**Significant at p <0.05*

Discussion

Hypertension, obesity, dyslipidemia and insulin resistance are characters of metabolic syndrome. The present study revealed an increased in systolic blood pressure, visceral fat weight,insulin resistance and total serum cholesterol in a HF-model simulating metabolic syndrome in human. Additionally, there were reduction in activities of glutathione peroxidase and catalase enzyme activities as antioxidant enzymes and in glutathione replenishing ability in hepatic cells of this model. On the other hand, there was a reduction in superoxide dismutase enzyme (SOD) level in erythrocyte lysates of this model. All these changes are reversed by treatment with single daily dose administration of G115 for 6 weeks of HF-fed albino rats. Korean red ginseng (KRG) also revealed its ability to overcome the condition of chronic low grade inflammation and insulin resistance, that accompanies metabolic syndrome [20].

Han, et al [21] reported that red ginseng significantly decreased systolic blood pressure in essential hypertension patients without antihypertensive medication.

The results of the present study are comparable to the findings reported with KRG that shows an improvement of insulin resistance and anti-hyperlipidemic effects [22,23].

Three herbs, ginseng, rhizoma coptidis (berberine, the major active compound) and bitter melon, are related to ginseng species. Ginseng extracts, which were prepared from root, rootlet, berry and leaf of American ginseng and Panax ginseng, also possess the ability to protect islets of Langerhans that release adequate amounts of insulin. All cells of human's body are properly responding to insulin (i.e. possess the property of insulin sensitization) that helps to maintain control on blood glucose levels without induction of hyperglycemia. It has anti-obesity and anti-oxidative activities as evidenced by many experimental studies. Loss of energy is enhanced by ginseng through thermogenesis. Ginsenosides are the major bioactive compounds for the metabolic activities of ginsengs and responsible for all mentioned biological activities of this herb. Panax ginseng also has anti-obesity and anti-dyslipidemia activities. The possible mechanism of action in such actions could be related to regulation of mitochondrial function, modification of glycolysis, activation of phosphorylation pathway, suppression of adipogenesis and suppression of low-density lipoprotein (LDL) receptor expression. Animal studies shows a possible protection of beta cells of pancreas, augmentation of insulin sensitivity and produce a protection against oxidative stress [8].

In conclusion, the results of the present study demonstrated that ginseng extract (G115) possesses a lowering effect on high systolic blood pressure, on total serum cholesterol and on insulin resistance with a significant enhancement of activities of important antioxidant enzymes in hepatic cells and RBCs lysates of albino rats exposed to a model of metabolic syndrome. These findings warrant further molecular investigations of action of G115 in metabolic syndrome.

Disclosure

The author reports no conflicts of interest in this work.

Acknowledgment

This research was officially supported by the Medical Research Service of the Ain Shams University. It was financially supported by the laboratory of the Pharmacology Department, Faculty of Medicine, Ain Shams University.

References

[1] Grundy S.M., Brewer H.B.Jr., Cleeman J.I., Smith S.C.Jr., Lenfant C. (2004) *Circulation*, 109(3), 433-438.

[2] Zambon J.P., Mendonça R.R., Wroclawski M.L., Karam Junior A., Santos R.D., Carvalho J.A., Wroclawski E.R. (2010) *Sao Paulo Med. J.,* 128, 137-40.

[3] Jeon B.H., Kim C.S., Park K.S., Lee J.W., Park J.B., Kim K.J., Kim S.H., Chang S.J., and Nam K.Y. (2000) *General Pharmacology: the Vascular System,* 35(3), 135-141.

[4] Yun S.N., Ko S.K., Lee K.H., Chung S.H. (2007) *Arch. Pharm. Res.*, 30, 587-95.

[5] Kim K., Kim H.Y. (2008) *J. Ethnopharmacol.*, 120 190-195.

[6] Kim H.S., Lee B.M. (2001) *J. Toxicol. Environ. Health*, A(63) 583-598.

[7] Sievenpiper J.L., Arnason J.T., Leiter L.A., Vuksan V. (2003) J. Am. Coll. Nutr., 22, 524-532.

[8] Yin J., Zhang H., Ye J. (2008) *Endocrine, Metabolic & Immune Disorders Drug Targets*, 8(2), 99-111.

[9] Xie J.T., Wang C.Z., Li X.L., Ni M., Fishbein A., Yuan C.S (2009) *Fitoterapia,* 80, 306-311.

[10] Faure P., Rossini E., Wiernspeger N., Richard M.J., Favier A., Halimi S. (1999) *Diabetes,* 48, 353-357.

[11] Rhee Y.H., Lee S.P., Honda K. and Inove S. (1990) *Psychopharmacol. Berl.*, 10(14), 486-488.

[12] Bunag R. (1973) *J. Appl. Physiol.*, 34, 279-282.

[13] Hansen P.A., Han D.H., Nolte L.A., Chen M., Holloszy J.O., (1997) *Am. J. Physiol.*, 273, 1704-1708.

[14] Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A., Treacher D.F., Turner R.C. (1985) *Diabetologia,* 28, 412-419.

[15] Rotruck J.T., Pope A.L., Ganther H.E., Swanson A.B., Hafeman D.G., Hoekstra W.G. (1973) *Science*, 179(4073), 588-590.

[16] Sinha K.A. (1972) *Ann. Biochem.*, 47, 389-394.

[17] Sato R., Goldstein J. and Brown M. (1993) *Proc. Natl. Acad. Sci.*, 90, 9261-9265.

[18] Saville B. (1958) *The Analyst*, 83, 670-672.

[19]Bradford M. (1976) *Anal. Biochem*., 72, 248-254.

[20]Kim K., Kim H.Y. (2008) *J. Ethnopharmacol.*, 120, 190-195.

[21]Han K.H., Choe S.C., Kim H.S., Sohn D.W., Nam K.Y., Oh B.H., Lee M.M., Park Y.B., Choi Y.S., Seo J.D., Lee Y.W. (1998) Am. J. Chin. Med., 26, 199-209.

[22]Lee H.J., Lee Y.H., Park S.K., Kang E.S., Kim H.J., Lee Y.C., Choig C.S., Parka S.E., AhnaC.W., Chaa B.S., Leeh K.W., Kimc K.S., Lima S.K., Leea H.C. (2009) *Metabolism*, 58, 1170-1177.

[23]Hasani-Ranjbar S., Nayebi N., Larijani B., Abdollahi M. (2009) *World J. Gastroenterol.*, 15, 3073-85.