CHAPTER VIII

8. ANTIDIABETIC EFFECT OF *Myxopyrum serratum* (MS) AND GELATIN ENCAPSULATED *Myxopyrum serratum* NANOPARTICLES (GMSN2) BY HIGH FAT DIET (HFD) INDUCED TYPE 2 DIABETES IN C57BL/6J MICE

8.1. INTRODUCTION

Obesity is the major risk factor for insulin resistance, which is generally caused by a western style high fat diet (HFD) and lack of physical activity (Zheng T *et al.*, 2012). HFD is known to increase body weight and visceral fat depots, induce alterations in carbohydrate and lipid metabolism leads to insulin resistance and an alteration in adipokines (Brown JL *et al.*, 2002). Excessive accumulation of lipids in nonadipose tissues such as liver, heart, skeletal muscle, kidney and pancreas contributes to the pathogenesis of fatty liver, heart failure and insulin resistance. Till now, numerous types of diabetic models are used for screening antidiabetic properties. These diabetic models are developed through several methods either through genetic or chemically induced diabetes (Zheng X *et al.*, 2012). Genetic models of diabetes known as db/db mouse and Zucker Diabetic Fatty (ZDF) rats which develop similar features to human type 2 diabetes (Clee SM & Attie AD, 2006). Besides, these rats are expensive to be used as diabetic models for pharmacological screening (Masiello P *et al.*, 2006). Meanwhile, development of diabetic rats following streptozotocin (STZ) injection also creates hyperglycemia (Mythili MD *et al.*, 2004). However, this method only develops insulin deficiency rather than insulin resistance (Shafrir E, 2003). Thus, several researchers have been investigating to find a better diabetic model for type 2 diabetes mellitus by modifying the existing method. Recently, many studies reported that rats induced with high fat
diet have developed similar situation as type 2 diabetes progress in humans. Diets containing high fat will cause insulin resistance in peripheral tissues due to lipotoxicity (Unger RH et al., 2010). High fat diet has successfully mimicked natural progress of diabetes development as well as metabolic features in human type 2 diabetes (Franconi F et al., 2008, Sahin K et al., 2007). Although there was a plenty amount of antidiabetic drugs available, the numbers of type 2 diabetes mellitus patients are still increasing constantly. There is a dramatic revival of interest in using natural sources for treating diabetes due to side effects of prolonged consumption of therapeutic drugs. More than 4000 plants have been studied and identified to have hypoglycemic effect through several mechanisms of antidiabetic activity. Some plants acts through either insulin secretagogues or insulin mimetic properties such as Momordica charantia, Aloe vera, and Allium sativum (Ravi kumar A et al., 2011, Malviya N et al., 2010 and Jung SH et al., 2009). Studies have reported that these plants have the ability to reduce blood glucose and improve insulin secretion (Dheer R and Bhatnagar P, 2010). Current study was designed to study the effect of M. serratulum extract (MS) and its nanoparticles(GMSN2) on the serum glucose level, antioxidant and carbohydrate metabolic enzyme profiles in high fat diet induced diabetes in C57BL/6J mice (Arjunan Sundaresan et al., 2012).

8.2. Materials

Apparatus

Glucometer and strips Accu-Check (Roche Diagnostics, Manheim, Germany), weighing scale (Mettle Pm 480 Delta ranged), intragastric oral canula, cages, 1 ml syringes, laboratory centrifuge
Drugs and Chemicals

Metformin, Sitagliptin (Surien Pharmaceuticals Limited, Chennai), Ethanolic extract of *Myxopyrum serratulum* (MS) and Gelatin encapsulated *Myxopyrum serratulum* nanoparticles (GMSN2).

Biochemical kits

Total cholesterol (TC), triglyceride (TG) kits were purchased from Accurex Biomedical Pvt. Ltd

Institutional Animal Ethical committee approval

Study protocol was approved by the Institutional Animal Ethics Committee (IAEC/XLVII/SRU/480/2016) of Sri Ramachandra University, Chennai, India and performed as per the guidelines of CPCSEA, India.

Animals

Male C57BL/6J mice of 4-weeks of age (20-22 g) were purchased from the central animal house facility of Biogen, Bangalore, India. Animals were housed (5 animals per polypropylene cage) in a room maintained under controlled temperature (22 ± 2°C) and humidity (55 ± 5%) with 12 h light and 12 h dark cycle at Centre for toxicological and developmental research, Sri Ramachandra University. Study protocol (Figure 8.2) were approved by the Institutional Animal Ethics Committee (IAEC/XLVII/SRU/480/2016) of Sri Ramachandra University, Chennai, India and performed as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All the animals had free access to commercially available normal pellet diet and water *ad libitum*. Animals were acclimatized to standard husbandry conditions for one week to eliminate the effect of stress prior to initiation of the experiments. Animal care and experimental
procedures were complied with the rules and regulations of the Institutional Animal Ethical Committee.

8.3. Experimental Induction of Type 2 diabetes

Type 2 diabetes was induced in male C57BL/6J mice by feeding a high-fat diet (HFD60 kcal % fat diet).

8.3.1. Composition of HFD

Research diets (60 kcal % fat diet) D12492, USA

Its caloric composition is

- 20 kcal% protein
- 20 kcal% carbohydrate
- 60 kcal% fat

Every gram of its ingredient contains 5.24 kcal calories, including 232 mg cholesterol from lard and blue dye.
Induction of Type 2 Diabetes

Briefly, six animals were fed normal diet (low fat diet); the other remaining animals were given high fat diet. Animals were weighed weekly and maintained on their respective diets for 10 weeks. Weekly body weight and daily food intake of mice were measured. After exposure to the diet for 10 weeks, animals were fasted overnight for a period of 8 h. Blood (0.5 ml) was withdrawn via the retro-orbital plexus under mild ether anesthesia and collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ ml of blood). The micro tubes were centrifuged at 4000 rpm at 4°C for 20 min to obtain clear plasma. The plasma was analyzed for glucose using Glucometer. Mice with blood glucose level of 190 mg/dL and above were considered to have developed insulin resistance and were subjected to intragastric administration of various doses of MS, GMSN2 and metformin (as mentioned in the experimental design) during 10th to 13th weeks. Normal diet feed group and High fat diet feed group animals received respective diets until the end of the study.
8.4. Experimental design

Diabetic Study

The Diabetic C57BL/6J male mice were randomly divided into 6 groups 10 animals for each group. Normal diet feed group served as normal control (6 animals).

**Group 1:** Mice received 0.5% sodium Carboxy methyl cellulose (CMC) along with standard pellet diet for 21 days

**Group 2:** Mice received 0.5% sodium CMC along with HFD diet for 21 days

**Group 3:** Mice received ethanolic extract of MS (400 mg/kg b.wt) along with HFD diet for 21 days

MS- *Myxopyrum serratulum*, GMSN2- Gelatin encapsulated *Myxopyrum serratulum* nanoparticles, Std- Metformin
Group 4: Mice received ethanolic extract of MS (800 mg/kg b.wt) along with HFD diet for 21 days

Group 5: Mice received GMSN2 (80 mg/kg b.wt) along with HFD diet for 21 days

Group 6: Mice received GMSN2 (160 mg/kg b.wt) along with HFD diet for 21 days

Group 7: Mice received Metformin (500 mg/kg b.wt) along with HFD diet for 21 days

The drugs were administered orally using a standard intragastric oral cannula once daily in the morning for the period of 21 days by mixing with the vehicle 0.5% sodium CMC. The mice were allowed to continue to feed on their respective diets until the end of the experiments. Weekly body weight and daily food intake of mice were measured.

8.4.1. Plasma preparation

At the end of the experimental period, after an overnight fast, mice were anesthetized and sacrificed. Blood (0.5 ml) was withdrawn via the retro-orbital plexus under mild ether anesthesia and collected in micro tubes previously filled with 10% EDTA solution (20 µl of 10% EDTA/ml of blood). The plasma was obtained from blood samples after centrifugation at 4000 rpm at 4°C for 20 min and stored at 4°C until analysis. The plasma was analyzed for glucose, triglyceride (TG) and total cholesterol (TC) in auto-analyzer using respective diagnostic kits.

8.4.2. Preparation of tissues for biochemical, histopathological studies

Immediately after mice were sacrificed, the liver was dissected out, cleaned and washed in ice-cold isotonic saline. Liver tissues were homogenized in a buffer (pH 7.0) to give 10% w/v homogenate. The homogenate was centrifuged at 1000 rpm for 10 min at 4°C in a cooling centrifuge. The supernatant was separated and used for
various biochemical estimations. A part of adipose tissues and liver were fixed in 10% neutral buffered formalin solution for histopathological examination.

8.5. Estimation of Biochemical parameters

Estimation of total protein content

The total protein content was measured by the method of Lowry et al., 1951.

Reagents:

1. Alkaline copper reagent:
   - Reagent A: 2% sodium carbonate was prepared using 0.1 N NaOH
   - Reagent B: 0.5 % copper sulphate was prepared in 1 % sodium potassium tartarate
   - Reagent C: 50 ml of reagent A was mixed with 0.5 ml of reagent B just before use

2. Folin's Ciocalteau reagent: Diluted with distilled water (1:2)

3. Stock standard: 100 mg of bovine serum albumin/100 ml of water

4. Working standard: 10 ml of the stock standard was diluted to 100 ml to get a working standard containing 0.1 mg/ml

Procedure

The assay mixture contained 0.1 ml of liver homogenate, 0.9 ml of NaOH (0.1 N) and 5.0 ml of alkaline copper sulphate reagent. The reaction mixture was incubated for 15 min at room temperature and then 0.5 ml of Folin Ciocalteau reagent was added. The reaction mixture was further incubated for 30 min at room temperature. The absorbance was measured at 660 nm. Bovine serum albumin was used as standard.
8.6. Estimation of Antioxidant parameters in liver homogenate

8.6.1. Estimation of reduced glutathione level

Reduced glutathione in the liver tissue was estimated by the method of Ellman G 1959. This method was based on the development of yellow color when 5, 5’ - dithio-bis 2-nitrobenzoic acid (DTNB) was added to compounds containing sulphydryl groups.

Reagents

1. Phosphate buffer: 0.2 M, pH 8.0
2. TCA: 5%
3. Ellman's reagent: 0.6 mM 12 mg 5,5’ dithio(bis)nitrobenzoic acid (DTNB) in 50 ml of Phosphate buffer
4. Standard glutathione solution: 100 mg GSH in 100 ml water
5. Working standard: Stock was diluted to get a concentration of 100 µg/ml.

Procedure

One ml of liver homogenate was added to 1.0 ml of TCA (5 %) and mixed thoroughly. The mixture was then centrifuged at 5000 rpm for 5 min at room temperature. The assay mixture consisted of 1.0 ml of this supernatant, 0.5 ml of Ellman’s reagent and 3 ml of phosphate buffer (0.2M, pH 8.0). The yellow color developed was immediately measured at 412 nm. A series of standards (20-100 µg) was treated in a similar manner along with a blank containing 1.0 ml of buffer. The amount of reduced glutathione was expressed as units/mg of protein for tissues

8.6.2. Estimation of glutathione peroxidase activity

Glutathione peroxidase (GPx) activity was assayed by the method of Rotruck et al., 1973. A known amount of enzyme preparation was allowed to react with H₂O₂
in the presence of reduced glutathione (GSH) for a specified time period. GPx utilize GSH for the decomposition of $H_2O_2$. After a specific time, the remaining GSH content was measured by Ellman’s method.

**Reagents**

1. Sodium azide 10mM: 16.27 mg in 25 ml distilled water
2. GSH (2mM) : 30.732 mg in 50 ml distilled water
3. $H_2O_2$ 1mM : 3ml in 100 ml distilled water
4. TCA : 10%
5. Tris HCl buffer : 0.4 M (pH 7)
6. DTNB 0.6mM : 12 mg in 50 ml Phosphate buffer (pH 8)

**Procedure**

The reaction mixture contained 1.0 ml of Tris HCl buffer (400 mM, pH 7), 0.5 ml of sodium azide (10 mM), 1.0 ml of K.EDTA, 0.5 ml liver homogenate, 0.5 ml glutathione (2 mM) and the reaction was started by addition of 1.0 ml of $H_2O_2$. The reaction mixture was incubated for 10 min at 37°C. The reaction was stopped by addition of 1.5 ml TCA (10%) and centrifuged for 5 min at 5000 rpm. GSH in the protein free filtrates was determined by mixing 1.0 ml of supernatant with 2.0 ml of saline and 1.0 ml of DTNB solution. The absorbance was read at 412 nm. Glutathione peroxidase activity is expressed as $\mu$M of GSH utilized/min/mg protein (U/mg protein). For control, 0.5 ml of Tris-HCl buffer was used in place of the liver homogenate.

**8.6.3. Estimation of superoxide dismutase**

Superoxide dismutase in the tissues was assayed by the method of Kakkar et al., 1984. The assay was based on the inhibition of the formation of NADH phenazine
methosulphate nitroblue tetrazolium formazan. The reaction was initiated by the addition of NADH. After incubation for 90 seconds, glacial acetic acid was added and stopped the reaction. The color developed at the end of the reaction was extracted into n-butanol layer and measured at 520 nm.

**Reagents**

1. Sodium pyrophosphate buffer: 0.025 M, pH 8.3
2. n-butanol
3. Phenazine methosulphate (PMS): 186 µM
4. Nitroblue tetrazolium (NBT): 300 µM
5. NADH: 780 µM

**Procedure**

The assay mixture contained 50 µl of homogenate, 0.3 ml of sodium pyrophosphate buffer, 25 µl of phenazine methosulphate, and 75 µl of nitroblue tetrazolium. The reaction was started by the addition of 75 µl NADH. After incubation at 30°C for 90 sec, the reaction was stopped by the addition of 250 µl glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 2 ml n-butanol. The mixture was allowed to stand for 10 min and centrifuged for 30 min. The n-butanol layer was separated; the color density of the chromogen in n-butanol was measured at 520 nm. The specific activity of the enzyme was expressed as Unit/mg of protein.

**8.6.4. Estimation of TBARS**

Thiobarbituric acid reactive substances (TBARS) were measured by method of Niehaus and Samuelson 1968. This method is based on the reactivity of
thiobarbituric acid (TBA) in acidic condition to generate pink colored chromophore which was read at 535 nm.

**Reagents**

1. TBA : 0.8 % in 1N HCl
2. BHT : 0.05% in methanol
3. Saline : 0.9% of NaCl

**Procedure**

Assay mixture containing 0.2ml of 10% homogenate, 0.8ml of saline, 0.5ml of BHT (Butylated hydroxyl toluene) and 3.5 ml of TBA. The mixture was kept in a boiling water bath for 90 min; after cooling, the tubes were centrifuged at 3000 rpm for 10 minutes and the supernatant was estimated. A series of standard solution in the concentration of 2-10 nmol was treated in a similar manner. The absorbance of the chromophore was read at 535 nm against a reagent blank. The values were expressed as nmol/mg of tissues

**8.6.5. Estimation of free fatty acid**

Free fatty acid was estimated by the method of Falholt et al., 1973

**Reagents**

1. Cu-TEA : Copper triethanolamine solution (Mixture of 25 ml of 0.1M copper nitrate, 5 ml of triethanolamine and 16.5 g of NaCl)
2. CHM : Cholorform 20: Heptane 15: Methanol 0.7
3. SDDC: Sodium Diethyl dithiocarbamate 0.1%
4. Silicilic acid : Silicilic gel G54 washed 3 times with 10% Hcl & dried
Procedure

Assay mixture containing 0.1 ml 10% homogenate, 1.5 ml of CHM & 50 mg of silicilic acid. The mixture was stirred vigorously and centrifuged at 3500 rpm for 10 min. 1 ml of supernatant was treated with 0.5ml of Cu-TEA and again centrifuged at 3000 rpm for 10 min. Free fatty acid was estimated by mixing 0.8ml of supernatant with 0.1 ml of 0.1% SDDC. The absorbance was read at 440 nm. The values were expressed as nmol/mg of tissues.

8.7. Estimation of hepatic glucose regulating enzyme activities

8.7.1. Assay of hexokinase D (glucokinase)

Hexokinase D was assayed by the method of Brandstrup et al., 1957.

Reagents

1. Glucose solution: 0.005 M
2. ATP solution: 0.72 M
3. Magnesium chloride solution: 0.05 M
4. Dipotassium hydrogen phosphate solution: 0.0125 M
5. Potassium chloride solution: 0.1 M
6. Sodium fluoride solution: 0.5 M
7. Tris-HCl buffer: 0.01 M, pH 8.0

Procedure

The reaction mixture in a total volume of 5.5 ml contained the following: 1.0 ml of 0.005 M glucose solution, 0.5 ml of 0.72 M ATP, 0.5 ml of 0.05 M MgCl₂, 0.5 ml of 0.0125 M dipotassium Hydrogen phosphate solution, 0.4 ml of 0.1M KCl, 0.1 ml of 0.5M NaF solution and 2.5 ml of Tris-HCl buffer (0.01M; pH 8.0). The mixture was pre incubated at 37°C for 15 min. The reaction was initiated by the addition of 1.0
1.0 ml aliquot of the reaction mixture was taken immediately (zero time) to tubes containing 1.0 ml of 10% TCA. A second aliquot was removed after 30 min of incubation at 37ºC and added to tubes containing 1 ml of 10% TCA. The precipitated protein was removed by centrifugation and the residual glucose in the supernatant was estimated by the o-toluidine method of Sasaki et al., 1972. The green color developed was read in a colorimeter at 620 nm. A reagent blank was run with each test. The difference between the two values gave the amount of glucose phosphorylated.

8.7.2. Assay of fructose 1,6-bisphosphatase

Fructose 1, 6-bisphosphatase was assayed by the method of Gancedo and Gancedo, 1971.

Reagents

1. Tris-HCl buffer: 0.1 M, pH 7.0
2. Substrate: Fructose 1,6- bisphosphate, 0.05 M
3. Magnesium chloride: 0.1 M
4. Potassium chloride: 0.1 M
5. EDTA solution: 0.001 M
6. TCA: 10%
7. Molybdic acid: 2.5% ammonium molydate in 3 N sulphuric acid
8. Aminonaphthol sulphonic acid (ANSA) reagent: 500 g of ANSA was dissolved in 195 ml of 15% sodium bisulphate and 5 ml of 20% sodium sulphite. The solution was filtered and stored in a brown bottle.
9. Phosphorus stock Standard (80 µg/ml): 35.1 mg of potassium dihydrogen phosphate was dissolved in 100 ml of distilled water
Procedure

The assay medium in a final volume of 2.0 ml contained 1.0 ml of Tris-HCl buffer (0.1 M, pH 7.0), 0.4 ml of substrate as fructose 1,6- bisphosphate (0.05 M), 0.1 ml of magnesium chloride (0.1 M), 0.2 ml potassium chloride (0.1 M), 0.1 ml of 0.001 M EDTA and 0.2 ml of homogenate. The incubation was carried out at 37°C for 15 min. The reaction was terminated by the addition of 2 ml of 10% TCA. The suspension was centrifuged and the phosphorus content of the supernatant was estimated according to the method described by Fiske and Subbarow (1925). To 1 ml of an aliquot of the supernatant, 0.3 ml of distilled water and 0.5 ml of ammonium molybdate (2.5% ammonium molybdate in 3 N sulphuric acid) were added. After 10 min, 0.2 ml of 1-amino-2-naphthol-4-sulphonic acid (ANSA) was added. The tubes were shaken well, kept aside for 20 min and the blue color developed was read at 620 nm.

8.7.3. Assay of glucose 6-phosphatase

Glucose 6-phosphatase was measured by the method of Koide and Oda, 1959.

Reagents

1. Citrate buffer: 0.1 M, pH 6.5
2. Glucose 6-phosphate: 0.01 M in distilled water
3. Ammonium molybdate solution: 2.5 g ammonium molybdate dissolved in 100 ml of 3N sulphuric acid
4. Aminonaphthol sulphonic acid (ANSA) reagent: 500 g of ANSA was dissolved in 195 ml of 15% sodium bisulphite and 5 ml of 20% sodium sulphite was added to it. The solution was filtered and stored in a brown bottle
5. Trichloroacetic acid (TCA): 10%
Procedure

The incubation mixture contained 0.3 ml citrate buffer (0.1 M, pH 6.5), 0.5 ml of 0.01 M glucose 6-phosphate and 0.2 ml liver tissue homogenate. This was incubated at 37°C for 1 h. 1 ml 10% TCA was added to the tubes to terminate the enzyme activity and then centrifuged. To 1 ml of the aliquot of supernatant, 1 ml of 2.5% ammonium molybdate and 0.4 ml ANSA were added. The blue color developed was read after 20 min at 620 nm. A tube devoid of the enzyme served as control.

8.8. Histopathological studies

Adipose tissues and liver from representative mice in each group were fixed in 10% neutral buffered formalin, embedded in paraffin and cut into thin section (5 µm) and mounted on slide glasses. Hematoxylin and eosin (H&E) staining was performed according to the standard protocol as described by Bancroft and Gamble, 2008. Processed tissues were deparaffinized with two changes of xylene for 2 min each, rehydrated with two changes of absolute 95% and 80% alcohols for 2 min each, followed by washing in running tap water for 5 min. Then, the tissues were stained with haematoxylin for 20 min and washed in running tap water. Differentiation with 1% acid alcohol was carried out for 10 s, followed by washing and bluing by dipping the tissues in ammonia water for 10 s. After a washing step, the tissues were counter stained with eosin Y for 2 min, dehydrated with increasing graded of alcohols for 2 min each, cleared with two changes of xylene for 2 min each and finally mounted with dibutyl phthalate xylene (DPX). The slides were examined under light microscopy in order to demonstrate the histopathological changes of adipose tissues, liver and photographed. All deviations from normal histology were recorded and compared with the corresponding controls.
8.9. Immunohistochemical studies of Adipose tissues

Primary and secondary antibodies were purchased from Abcam Inc, Cambridge, MA, USA. Adipose tissues of various groups were trimmed, dehydrated in series of graded alcohol, embedded in paraffin wax and 4 μm thickness of tissue section were prepared. Slides were deparaffinized in xylene and rehydrated through descending grades of ethanol. Heat induced antigen retrieval was performed by microwaving paraffin embedded sections of adipose tissues (4 μm) in sodium citrate buffer (10 mM, pH 6.0). Endogenous peroxidase quenching was done by incubation with 0.3 % H2O2 in methanol for 30 min, blocked with 5 % normal goat serum in 1 % BSA for 30 min and incubated overnight at 4°C with primary antibodies (Anti -IL6 anti body (ab6672), Anti-TNF alpha antibody (ab6671)). Sections were then incubated consecutively with secondary anti-body (Goat Anti-Rabbit IgG H&L (HRP) (ab97051)) for 30 min at room temperature. The sections were then incubated with streptavidin-HRP polymer conjugate for 30 min at room temperature. Immunoreactivity was developed by stained with DAB (3, 3’-diaminobenzidine) chromogen for 15-20 min and counterstained with hematoxylin for 30 sec. The sections were dehydrated in alcohol, xylene and then mounted in DPX (Distrene - Plasticiser -Xylene) and photographed (Dinh C et al., 2015).

8.10. Statistical Analysis

The collected data’s of in vivo results were analysed with IBM. SPSS statistics software 23.0 Version. To describe about the data descriptive statistics mean & SEM were used. To find the significant difference between the bivariate samples in paired groups the Wilcoxon signed rank test was used. For the multivariate analysis in repeated measures the Friedman test was used. In both the above statistical tools the
probability value 0.05 is considered as significant level and 0.01 is considered as highly significant.

8.11. Results

In type 2 diabetic patients the elevated plasma glucose levels was caused by several factors. The most important factors are the pancreatic β cells failure to produce and release enough quantity of insulin and insulin resistance. Recently, from many studies it has been proved that high fat diet to C57BL/6J mice develops insulin resistance and this model is suitable for the investigation of antidiabetic effect of drugs. Since this model imitates the similar pathogenesis, clinical features and natural history of the diseases as humans. Therefore HFD induced diabetes in C57BL/6J mice was selected for the present study.

The metabolic syndrome type 2 diabetes was induced by HFD feeding in male C57BL/6J mice. The degree of insulin resistance and alterations in the lipid metabolic parameters mainly depends on the duration of HFD feeding, type of the diets and lipid contents. HFD feeding to rodents for more than 8 weeks could develop metabolic abnormalities including hyperglycemia, overweight, dyslipidemia, hypertension and insulin resistance.

The antidiabetic effect of MS and GMSN2 was evaluated by HFD induced Diabetes in C57BL/6J mice. After the acclimatization period, animals were fed with HFD except normal control group, fed normal pellet diet over a period of 10 weeks. The mice utilized the HFD over a period of 10 weeks developed the drastic weight gain and hyperglycemia compared to the control group. After the induction of diabetes, the diabetic mice were randomized into 6 groups for treatment with different
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doses of MS, GMSN2 and Metformin for 21 days. During the entire induction and
treatment period daily feed intake and weekly body weight was measured.

8.11.1. Effect of MS and GMSN2 on Feed intake

During the first 2 weeks of induction period, feed intake of HFD group was
higher, after that there is a reduction of feed intake in HFD group (Figure 8.3a). After
treatment with MS (400 mg/kg b.wt, 800 mg/kg b.wt) and GMSN2 (80 mg/kg b.wt,
160 mg/kg b.wt) and metformin (500 mg/kg b.wt) showed there was little difference
in the feed intake but no significant difference between drugs treated groups (Figure
8.3b). In the normal control group the feed intake was more than HFD group and this
level was maintained throughout the entire study period.

8.11.2. Effect of MS and GMSN2 on Body weight

Body weight was measured weekly once in normal diet fed and HFD fed
groups from 0 week to 13th week to assess the effect of MS and GMSN2 in diabetic
mice. In the successive feeding of HFD for 10 weeks, the animals gained weight
continuously which was significantly more than the normal diet fed group (Figure
8.4a). After the treatment was started with different doses of MS (400 mg/kg b.wt,
800 mg/kg b.wt), GMSN2 (80 mg/kg b.wt, 160 mg/kg b.wt) and metformin (500
mg/kg b.wt) illustrated that decrease in the body weight in all treated groups form 11
week to 13 week, but there is no significant difference between drug treated groups
(Figure 8.4b).
Figure 8.3: Effect of MS and GMSN2 on Feed intake of diabetic C57BL/6J mice
during Induction (a) and treatment period (b)
8.11.3. Effect of MS and GMSN2 on Blood glucose level

After the induction period, diabetic control mice showed significantly increased plasma glucose level when compared to normal control group. In normal control group the blood glucose level was normal throughout the study. Oral administration of ethanolic extract of MS (400 mg/kg & 800 mg/kg b.wt) along with high fat diet showed marked and significant reduction of plasma glucose level in a dose dependent manner over the period of 21 days treatment compared to diabetic control group (Table 8.1). Oral administration of GMSN2 at 80 mg/kg and 160 mg/kg b.wt produced a significant decrease in fasting blood glucose in comparison with MS.
at 400 mg/kg and 800 mg/kg b.wt. GMSN2 at 160 mg/kg reduces the blood glucose levels in HFD mice similarly with the same effectiveness as that observed with MS at higher dose (800 mg/kg). Thus it confirming the prepared nanoparticles are effective at one fifth of the extract dose in reducing the blood glucose level (Figure 8.5a). Standard drug metformin (500 mg/kg b.wt) treated group also showed significant reduction in the blood glucose level compared to diabetic control group.

8.11.4. Effect of MS and GMSN2 on Total cholesterol, Triglyceride and Free fatty acid levels in C57BL/6J mice

Total cholesterol and triglycerides levels were significantly increased in the HFD group compared with the normal control group over the period of ten weeks of HFD feeding. After administration of MS at higher dose (800 mg/kg b.wt) the levels of triglycerides, cholesterol and free fatty acids were significantly reduced from 184 to 119.97, 218.73 to 118.62 and 152.62 to 80.91 respectively. The lower dose of MS at 400 mg/kg b.wt showed significant reduction in plasma triglycerides (184 to 136.41), cholesterol (218.73 to 155.27) and free fatty acids (152.69 to 113.20) compared to diabetic control group. GMSN2 treatment at 80 mg/kg & 160 mg/kg doses also found to significantly decrease the triglycerides, total cholesterol and free fatty acid levels in HFD induced diabetes mice compared with diabetic control groups. GMSN2 at 160 mg/kg (one fifth of the extract dose) reduced all these parameters near to normal levels. These reductions were not significant when compared to MS treated groups but significant compared to diabetic control (Figure 8.5 b, c & d). MS at 400 mg/kg treated group and GMSN2 80 mg/kg (one fifth dose of MS 400 mg/kg) treated group showed similar effect in reducing the triglycerides, cholesterol and free fatty acids. This confirms that the gelatin encapsulated *Myxopyrum serratum* nanoparticles (GMSN2) at 80 mg/kg produces
antihypertriglyceridemic and antihypercholesteromic effect in High fat diet induced diabetes in C57BL/6J mice.

**Figure 8.5: Effect of MS and GMSN2 on Blood glucose (a), total cholesterol (b), triglycerides (c) and free fatty acid (d) levels of diabetic C57BL/6J mice**

Values are means ± SEM (n=6), Wilcoxon signed rank followed by Friedman test was used. * P ≤ 0.01 compared with normal control; ** P≤0.01 compared with Diabetic control, # P ≤ 0.05 compared with diabetic control
8.11.5. Effect of MS and GMSN2 on Hepatic glucose regulating enzyme activities

The activities of glycolytic and gluconeogenic enzymes in the liver of control and experimental animals are illustrated in table 8.3. A significant decrease in the hexokinase activity was found in the vehicle treated diabetic control group. On the other hand, there was a significant increase in the glucose-6-phosphatase and fructose 1,6 bisphosphatase activity in vehicle treated diabetic control group compared to vehicle treated normal control group. After treatment with MS and GMSN2 at different doses for 21 days, the altered levels were restored to normal levels. Oral supplementation of MS (400 mg/kg and 800 mg/kg b.wt) to C57BL/6J diabetic mice for 21 days significantly increases the glycolytic enzyme activity and decreases the gluconeogenic enzyme activity. While oral administration of GMSN2 (80 mg/kg and 160 mg/kg) also reverse the activities of the above enzymes significantly near to normal level. HFD mice treated with GMSN2 at 80 mg/kg for three weeks significantly increases the glycolytic enzyme activity and decreases the gluconeogenic enzyme activity in liver. This activity was similar to the effect produced by MS at higher dose of 400 mg/kg. This shows that GMSN2 in the lesser doses (one fifth of the extract) produces significant effect on hepatic glucose regulating enzyme activities when compared with extract. Metformin treated group further significantly reverse the altered enzyme levels towards normal levels (Figure 8.6). This effect may be due to the presence of tannins, saponins, flavonoids and phenols present in the extract which could act synergistically or independently in enhancing the activity of carbohydrate enzymes.
Figure 8.6: Effect of MS and GMSN2 on Hepatic glucose regulating enzyme activities of diabetic C57BL/6J mice

Values are means ± SEM (n=6), Wilcoxon signed rank followed by Friedman test was used. * P ≤ 0.01 compared with normal control; ** P≤0.01 compared with Diabetic control, # P ≤ 0.05 compared with diabetic control
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8.11.6. Effect of MS and GMSN2 on antioxidant activities

The alteration in the activities of antioxidant enzymes in the liver of diabetic control group and other experimental groups are portrayed in table 8.2. A significant reduction was observed in the activities of superoxide dismutase, reduced glutathione and glutathione peroxidase in the diabetic animals compared to those in the normal animals. Supplementation of different doses of MS (400 mg/kg and 800 mg/kg b.wt) among diabetic mice brought back the alterations in the antioxidant enzyme SOD, GPx and GSH levels with maximum effect observed at 800 mg/kg b.wt as compared to other dose. Treatment with GMSN2 (80 mg/kg and 160 mg/kg b.wt) reverses the levels of SOD, GPx and GSH more potentially than MS. More significant effect was observed at 160 mg/kg (Figure 8.7). It is important to note that MS and GMSN2 produce the antioxidant effect in a dose dependent manner. After 21 days treatment with low dose (80 mg/kg) of GMSN2, the antioxidant enzymes level in liver were found to be increased significantly and this is in comparison with the effect produced by MS 400 mg/kg. This confirms that the low dose of GMSN2 was found to be effective for increasing the antioxidant enzyme status in HFD mice.

8.11.7. Effect of MS and GMSN2 on Lipid peroxidation

Table 8.2 displayed the effect of MS and GMSN2 on the levels TBARS in liver. The HFD fed diabetic control group exhibited significant increase in the TBARS levels compared to normal control mice. Lipid peroxidation level was significantly reduced after treatment with MS (400 mg/kg and 800 mg/kg b.wt) and GMSN2 (80 mg/kg & 160 mg/kg b.wt) when compared to the vehicle treated diabetic control group (Figure 8.7). HFD mice treated with MS at 800 mg/kg exhibited more significant effect in increasing TBARS levels in liver when compared to HFD mice treated with 400 mg/kg of MS. Similarly, GMSN2 at 160 mg/kg b.wt produces more
significant effect in altering lipid peroxidation levels compared to GMSN2 at 80 mg/kg. In metformin treated group, TBARS level was significantly reduced and near to normal level.

**Figure 8.7: Effect of MS and GMSN2 on antioxidant enzyme activities and Lipid peroxidation levels of diabetic C57BL/6J mice**

Values are means ± SEM (n=6), Wilcoxon signed rank followed by Friedman test was used. * P ≤ 0.01 compared with normal control; ** P≤0.01 compared with Diabetic control, # P ≤ 0.05 compared with diabetic control
Table 8.1: Effect of MS and GMSN2 on plasma glucose, total cholesterol and triglycerides levels in HFD induced Diabetes in C57BL6J mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose mg/dl</th>
<th>Total Cholesterol mg/dl</th>
<th>Triglycerides mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>80.86±1.80</td>
<td>91.32±3.62</td>
<td>72.03±6.21</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>195.86±5.27*</td>
<td>218.73±8.92*</td>
<td>184±6.66*</td>
</tr>
<tr>
<td>MS (400mg/kg b.wt)</td>
<td>141.00±7.23**</td>
<td>155.27±5.05**</td>
<td>136.41±3.53#</td>
</tr>
<tr>
<td>MS (800mg/kg b.wt)</td>
<td>117.00±11.63**</td>
<td>118.62±4.78**</td>
<td>119.97±2.08**</td>
</tr>
<tr>
<td>GMSN2 (80mg /kg b.wt)</td>
<td>148.71±3.42**</td>
<td>164.92±3.32**</td>
<td>148.26±4.28**</td>
</tr>
<tr>
<td>GMSN2 (160mg /kg b.wt)</td>
<td>125.29±5.40**</td>
<td>122.92±1.96**</td>
<td>106.09±3.09**</td>
</tr>
<tr>
<td>Metformin (500 mg/kg b.wt)</td>
<td>97.29±5.24**</td>
<td>112.96±2.83**</td>
<td>94.48±3.73**</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n=6), Wilcoxon signed rank followed by Friedman test was used.* P ≤ 0.01 compared with normal control; ** P≤0.01 compared with Diabetic control, # P ≤ 0.05 compared with diabetic control
Table 8.2: Effect of MS and GMSN2 on liver antioxidant enzymes and free fatty acid levels in HFD induced Diabetes in C57BL6J mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD Units/mg protein</th>
<th>GPX Units/mg protein</th>
<th>GSH Units/mg protein</th>
<th>TBARS nMol/mg tissue</th>
<th>FFA nMol/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>13.96± 0.16</td>
<td>8.45± 1.01</td>
<td>165.69± 3.00</td>
<td>76.98± 3.69</td>
<td>49.65± 4.11</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>4.27± 0.28*</td>
<td>4.85± 2.14*</td>
<td>69.45± 4.83*</td>
<td>172.78± 15.94*</td>
<td>152.69± 5.19*</td>
</tr>
<tr>
<td>MS (400mg/kg)</td>
<td>6.44± 0.07**</td>
<td>6.12± 2.13**</td>
<td>91.93± 8.22#</td>
<td>121.28± 3.69</td>
<td>113.20± 3.64**</td>
</tr>
<tr>
<td>MS (800mg/kg)</td>
<td>10.24± 0.12**</td>
<td>8.17± 2.01**</td>
<td>138.0± 5.52**</td>
<td>90.46± 1.66#</td>
<td>80.91± 5.24**</td>
</tr>
<tr>
<td>GMSN2 (80mg /kg)</td>
<td>7.13± 0.17**</td>
<td>5.54± 3.05**</td>
<td>102.08± 8.68**</td>
<td>135.73± 5.21</td>
<td>110.95± 5.36**</td>
</tr>
<tr>
<td>GMSN2 (160mg /kg)</td>
<td>9.30± 0.38**</td>
<td>7.90± 4.15**</td>
<td>133.72± 4.60**</td>
<td>106.44± 9.82#</td>
<td>87.67± 13.01**</td>
</tr>
<tr>
<td>Metformin (500 mg/kg)</td>
<td>7.09± 0.44**</td>
<td>6.94± 0.01**</td>
<td>141.51± 1.72**</td>
<td>95.65± 7.08#</td>
<td>76.20± 9.98**</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n=6), Wilcoxon signed rank followed by Friedman test was used. * P ≤ 0.01 compared with normal control; ** P≤0.01 compared with Diabetic control, # P ≤ 0.05 compared with diabetic control
Table 8.3: Effect of MS and GMSN2 on liver carbohydrate enzyme levels in HFD induced Diabetes in C57BL6J mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase (mM of Glu phosphorylated/mg protein)</th>
<th>Fructose 1,6 bisphosphatase (mM Pi released / mg protein)</th>
<th>1,6 Glucose phosphatase (mM Pi released / mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.31±0.01</td>
<td>0.17±0.03</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>0.15±0.04*</td>
<td>0.60±0.10*</td>
<td>0.75±0.15*</td>
</tr>
<tr>
<td>MS (400mg/kg)</td>
<td>0.19±0.13#</td>
<td>0.40±0.02#</td>
<td>0.51±0.09</td>
</tr>
<tr>
<td>MS (800mg/kg)</td>
<td>0.25±0.01**</td>
<td>0.25±0.16**</td>
<td>0.32±0.05**</td>
</tr>
<tr>
<td>GMSN2 (80mg /kg)</td>
<td>0.17±0.05</td>
<td>0.45±0.12#</td>
<td>0.56±0.11</td>
</tr>
<tr>
<td>GMSN2 (160mg /kg)</td>
<td>0.21±0.09**</td>
<td>0.24±0.05**</td>
<td>0.37±0.09**</td>
</tr>
<tr>
<td>Metformin (500 mg/kg)</td>
<td>0.28±0.11**</td>
<td>0.19±0.11**</td>
<td>0.28±0.04**</td>
</tr>
</tbody>
</table>

Values are means ± SEM (n=6), Wilcoxon signed rank followed by Friedman test was used. * P ≤ 0.01 compared with normal control; ** P≤0.01 compared with Diabetic control, # P ≤ 0.05 compared with diabetic control

8.11.8. Effect of MS and GMSN2 on Histopathology of adipose tissues and Liver:

Adipose tissues and liver were evaluated for general histopathological changes by haematoxylin and eosin staining method. Figure 8.8 shows the effect of MS and GMSN2 on adipose tissues histopathological variations in HFD mice. It has been reported that the insulin responsiveness of adipose tissue depends on the size of adipocytes. That is, larger adipose cells are less sensitive to insulin, HFD mice showed an expansion of adipocytes with increased inflammation when compared to Normal control group. The crown-like structures appeared in adipose tissue samples of HFD mice representing an accumulation of macrophages around dead adipocytes.
MS (400 mg/kg) and GMSN2 (80 mg/kg) treated group showed minimal expansion of adipocytes with mild inflammation. MS (800 mg/kg), GMSN2 (160 mg/kg) and metformin treated group showed restoring of normal size of adipocytes without any inflammation when compared to that of diabetic control group.

Haematoxylin and eosin staining of liver revealed that HFD mice developed high degree of hepatic steatosis. Diabetic mice treated with MS at 400 mg/kg and GMSN2 at 80 mg/kg showed the presence of severe perivascular mononuclear cell infiltration and biliary hyperplasia in the liver. Mild mononuclear cell infiltration in liver was observed in the MS at 800 mg/kg and GMSN2 at 160 mg/kg treated group. Metformin treated group restored the normal architecture of the liver with mild mononuclear cell infiltration (Figure 8.9).

8.11.9. Immunohistochemical studies of Adipose tissues

In obese individuals, during the development and progression of obesity, the adipose tissues possess hypertrophic adipocytes and produce various proinflammatory mediators, including cytokines, chemokines and hormones (Ouchi N et al., 2011). The mediators either assemble in or leak out of adipose tissues, leading to the enhancement of inflammation levels in local tissues and/or sera. Finally, the pathological events further exacerbate fat deposition and obesity-related insulin resistance. Interleukin-6 and TNF-alpha are among the best known and well investigated cytokines produced in adipose tissues. The expression of TNF-alpha in adipose tissue is increased in conditions of obesity and closely correlates with hyperinsulinemia. Moreover weight loss promotes a significant decrease in the TNF-alpha expression in adipose tissues. It could also be demonstrated that TNF-alpha inhibits insulin-dependent glucose uptake in human adipocytes, which correlates a
significant reduction of the density of insulin-regulated glucose transporter-4 protein at the cellular membrane. Hence, we examined the expression levels of important pro-inflammatory cytokines (TNF-α and IL-6) involved in fat deposition and insulin resistance by immunohistochemical studies. The intense brown staining in the adipose tissues represents the localization of large amount of inflammatory cytokines TNF-α and IL-6. This was seen in the diabetic control group. The expression of these cytokines were reduced to a greater extent in the MS (800mg/kg), GMSN2 (160mg/kg) treated group compared to that of metformin treated group. Whereas the minimal expression was observed in the MS (400 mg/kg), GMSN2 (80 mg/kg) treated group (Figure 8.10 and 8.11). GMSN2 significantly reduced the expression of TNF-α and IL-6 to a greater extent at low dose (160 mg/kg) when compared to that MS at (800 mg/kg). Histo and Immunohisto pathology results suggest that GMSN2 at lower doses (80mg/kg and 160mg/kg) having potential effect in preventing obesity-induced adipose tissue systematic inflammation.
Figure 8.8: Histopathological images of Adipose tissues

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Diabetic control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inflammation (40x)</td>
<td>Inflammation (40x)</td>
</tr>
<tr>
<td>MS (400mg/kg b.wt)</td>
<td>MS (800mg/kg b.wt)</td>
</tr>
<tr>
<td>Moderate inflammation (40x)</td>
<td>Mild inflammation (40x)</td>
</tr>
</tbody>
</table>
GMSN2 (80 mg/kg b.wt)  
**Moderate inflammation (40x)**

GMSN (160 mg/kg b.wt)  
**No inflammation (40x)**

Metformin (500 mg/kg b.wt)  
**No inflammation (40x)**
Chapter VIII

Figure 8.9: Histopathological images of Liver

Normal Architecture (10x)

Diabetic Control

Severe Hepatic Steatosis (10x)

MS 400 mg/kg

MS 800 mg/kg

Biliary hyperplasia and severe perivascular mononuclear cell infiltration (40x)

Diffuse mononuclear cell infiltration in the perivascular area (40x)
GMSN2 80 mg/kg

Mononuclear cell infiltration and biliary hyperplasia (40x)

GMSN2 160 mg/kg

Moderate centrilobular vacuolar degeneration (10x)

Metformin 500 mg/kg

Normal architecture with mild mononuclear cell infiltration
Figure 8.9: Effect of MS and GMSN2 on the expression of TNF-α in adipose tissue
Figure 8.10: Effect of MS and GMSN2 on the expression of IL-6 in adipose tissue
8.12. DISCUSSION

Insulin resistance is the most common clinical manifestations among several clinical disorders including type 2 diabetes and cardiovascular disease (Schaffer & Mozaffari, 1996). It is a central feature of many physiological and pathological states and leads to wide variety of biochemical defects in the insulin action cascade (Virkamaki A et al., 1999). Two major key aspects of type 2 diabetes is inadequate glucose uptake in major glucose utilizing tissues; and visceral obesity, these are manifested by insulin resistance. In fact, 75-80% of mortality rate in type 2 diabetic patients are due to a combination of coronary heart disease, peripheral vascular disease and cerebrovascular diseases. Insulin resistance and lipid disorder can be induced by a High fat diet feeding in C57BL/6J mice (Cong WN et al., 2008). The genetic basis of this model similar to type 2 diabetes in humans (Surwit et al., 1988). The severity of diabetes depends on duration of HFD feeding and the lipid contents in the diet. Several research studies have provided evidence that patients with type 2 diabetes have several abnormalities in their blood, lipid profiles and various other biochemical parameters (Wilson 2005). In the present study, the C57BL/6J mice fed the HFD over a period of 10 weeks showed statistically significant drastic weight gain and increased blood glucose level than normal control group. HFD consumption led to obesity as it facilitates fat storage in muscle rather than oxidation and assists the development of a positive energy balance leading to an increase in visceral fat deposition; this led to abdominal obesity. After treatment with MS and GMSN2 for 21 days leads to reduced body weight compared to diabetic control group with slight variation in the food consumption indicating that the effect is not caused by a decrease of calorie intake. The minimal reduction of body weight gain observed in MS and GMSN2 treated mice may be due to the influence of MS and GMSN2 on both
carbohydrate and lipid metabolism. Our results also showed that treatments with MS and GMSN2 lowered blood glucose, triglycerides and total cholesterol contents in the HFD mice, suggesting that MS and GMSN2 could effectively lower HFD induced hyperglycemia and hyperlipidemia. Insulin resistance is not only associated with hyperinsulinemia and hyperglycemia but also with other disorders such as abnormal lipid profile disturbances, which was improved after treatment with MS and GMSN2 and metformin. Reduction in the plasma glucose level is attributed to mechanism that involves insulin action rather than insulin secretion. Free fatty acid, triglycerides and total cholesterol contents were significantly higher in the diabetic control mice. Higher level of free fatty acid is known to contribute to insulin resistance by inhibiting insulin signaling and also by suppressing pancreatic insulin secretion (Arner P, 2003). Elevation in FFA level in obesity and type 2 diabetes mellitus has been accredited to non-esterification in the adipose tissue and the consequent escape from the adipose tissue to the local tissues or plasma (Riemens SC et al., 2000). The high TG level in the plasma can be related with increased TG synthesis in hepatic tissues using the freely available FFA which leads to hypertriglycerdemia in turn can contribute to development of insulin resistance (Grundy M, 1999). The level of TC in plasma is the major determinant of the risk of cardiovascular disease. Insulin resistance is not only associated with hyperglycemia but also with abnormal lipid profile disturbances, which was significantly improved after treatment with MS and GMSN2. However, oral administration of GMSN2 at 160 mg/kg for 3 weeks significantly restored the blood glucose and lipid profiles to near normal levels in HFD mice. Moreover, the improvement impact of GMSN2 at low dose on biochemical parameters was similar to MS with high dose. These results were correlates with the study reported by Ebtihal F. Alamoudi et al., (2014).
Previous literatures revealed that the changes in the hepatic glucose regulating enzyme activities in the experimental animals are mainly responsible for the hypoglycemic effect of medicinal plants (Jayaprakasam et al., 2006). In general, increased hepatic glucose production and decreased hepatic glycogen synthesis and glycolysis, are the major changes in type 2 diabetes. These may be due to the consequence of the low glucokinase activity and high glucose-6-phosphatase and fructose-1,6 bisphosphatase activity in a diabetic state (Guignot and Mithieux, 1999). Hepatic glucokinase enzyme is a potential target for the pharmacological treatment of type 2 diabetes because it has a major effect on glucose homeostasis. An increase of hepatic glucokinase activity can cause increased utilization of blood glucose for energy production or glycogen storage in the liver (Postic et al., 1999). Glucose 6 phosphatase and fructose-1,6-bisphosphatase are important enzymes in regulating gluconeogenic pathway. The activity of glucose 6 phosphatase and fructose 1, 6 bisphosphatase were found to be increased in the liver of diabetic mice (Saxena AK et al., 1992). Glucose-6-phosphatase catalyzes the last enzymatic reaction, which is also included in gluconeogenesis reactions and confers the liver to release glucose in the blood. Fructose-1,6-phosphatase is an important regulatory enzyme in the gluconeogenic pathway (Minnassian et al., 1994) and catalyzes the rate limiting step of fructose-1,6-bisphosphate to fructose-6-phosphate. In diabetes, the activity of fructose-1,6-phosphatase is observed to be increased in the liver and results in decreased glycolytic flux. Due to their strategic positions in the liver glucose metabolism, both these enzymes are supposed to be the target of important regulatory mechanisms of hepatic glucose production (Foster et al., 1997).
The present study demonstrated that hepatic glucose-6-phosphatase activity in diabetic control mice was significantly higher than that of normal rats and the oral administration of MS and GMSN2 showed an inhibition of gluconeogenesis by inhibiting the activity of glucose 6 phosphatase and fructose 1, 6 bisphosphatase. The increased activities of these gluconeogenic enzymes in diabetic mice were decreased near to normal levels after the oral administration of MS and GMSN2 to experimental diabetic mice (Baquer NZ et al., 1998). Gelatin encapsulated *Myxopyrum serratum* nanoparticles restored the carbohydrate metabolizing enzyme levels in liver with the dose of 80 mg/kg and 160 mg/kg. The study confirms that 80 mg/kg dose of GMSN2 was sufficient to restore the glycolytic and gluconeogenic enzymes in comparison to the MS at 400 mg/kg. These results suggest that *Myxopyrum serratum* encapsulation in gelatin nanoparticles allows for a dose reduction for the same pharmacological antidiabetic effect when compared to its ethanolic extract. A similar phenomenon was described by Deepa V et al., (2012), where the nanoencapsulated *Enicostemma littorale* had greater antidiabetic activity than their conventional extract form.

TBARS is the most commonly used markers of lipid peroxidation in plasma. Present study results showed that HFD significantly increased the TBARS levels in liver. This increased production of TBARS is due to the superoxide radical overload, subsequent increase in the production of hydrogen peroxide (Bopanna K.N et al., 1997). After treatment with MS and GMSN2 for 21 days, the lipid peroxidation level was significantly reduced. The antioxidant enzymes such as SOD, GSH and GPx have the ability to eliminate ROS and remove cytotoxic peroxides in mammalian systems. The inhibition of these enzyme activities produces many degenerative diseases. GSH is essential for the cellular antioxidant defense response and acts as an essential cofactor for antioxidant enzymes, SOD catalyzes the dismutation of the highly
reactive superoxide to the less reactive hydrogen peroxide, GPx reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water. Depletion in the activities of these antioxidant enzymes can be owed to an enhanced radical production (Venkateswaran S et al., 2002). Our result showed that HFD decreased the activities of SOD, GSH and GPx in liver which suggest that HFD induced an imbalance in the antioxidant defense systems. The additive effect of the MS and GMSN2 increasing SOD, GSH and GPx activities could be partially explained by their combined action on scavenging free radicals. The antioxidant enzyme level in liver was increased by the MS treatments at dose 400 mg/kg and 800 mg/kg. On the contrary, GMSN2 at low dose 80 mg/kg increased the antioxidant enzyme as same as that of MS at 400 mg/kg during the treatment period. The present study revealed that GMSN2 at low dose 80 mg/kg (one fifth of the extract dose 400mg/kg) was more active than MS in the regulation of antioxidant enzyme levels.

Histopathological studies of adipose tissues treated with MS and GMSN2 revealed that a reduction in the number of large-sized adipocytes and an increase in the number of small-sized adipocytes are correlated to improved insulin sensitivity and reduced obesity-induced insulin resistance (Okuno A et al., 1998). In obesity, adipose tissue becomes inflammed, demonstrated by the infiltration of immune cells (macrophages), which leads to increased production of proinflammatory cytokines (D. Konrad and S. Wueest 2014). Present study, demonstrated the anti-inflammatory property of *Myxopyrum serratum* (MS) and Gelatin encapsulated *Myxopyrum serratum* nanoparticles (GMSN2) through suppression of proinflammatory cytokines.
Present study revealed that HFD mice treated with MS at 800 mg/kg and GMSN2 at 160 mg/kg for 21 days significantly reduces blood glucose, body weight; increases antioxidant enzymes levels, restore the carbohydrate metabolic enzymes levels and reduces the expression of proinflammatory cytokines in adipose tissue. MS at 800 mg/kg treated group and GMSN2 at 160 mg/kg treated group showed similar antidiabetic effect in high fat diet induced diabetes in C57BL/6J mice. This effect was attributed by converting Myxopyrum serratulum (MS) to Gelatin encapsulated Myxopyrum serratulum nanoparticles (GMSN2) and the dose required to produce antidiabetic effect was found to be significantly reduced, it may be due to increased bioavailability in nanoformulation.
8.13. REFERENCES


17. Foster J. D., Pederson B. A., Nordlie R. C. Glucose-6-phos-phatase structure, 
314–332.
the available experimental models of type 2 diabetes appropriate for a gender 
19. Gancedo J. M., Gancedo C. Fructose 1, 6-bisphophatase, phospho 
fructokinase and glucose 6-phosphate dehydrogenase from fermenting yeast 
20. Grundy M. Hypertriglyceridermia, insulin resistance and metabolic syndrome. 
21. Guignot L., Mithieux G. Mechanisms by which insulin associated or not with 
glucose, may inhibit hepatic glucose production in the rat. Am. J. Physiol. 
1999; 277: 984–989.
22. Jayaprakasam B., Olson L. K., Schutzki R. E., Tai M. H., Nair G.M. 
Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 
mice by anthocyanins and ursolic acid in Cornelian cherry (Cornus mas). J. 
activities of tanshinones, diterpene compounds of the root of Salvia 
25. Koide H., Oda T. Pathological occurrence of glucose 6-phosphatase in serum 


42. Shafrir E, Diabetes in animals: contribution to the understanding of diabetes by study of its etiopathology in animal models, in Diabetes Mellitus, D. Porte,


