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Effect of the Mixture of *Pueraria lobata* and *Sorbus commixta* Extract on the Alcohol-induced Hangover in Rats

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Abstract – Pueraiae Radix (PR), Pueratia Folium (PF) and *Sorbus commixta* (SC) mixture, namely GS-SP (PR (1)/PF (2)/SC (0.5): *v/v/v*) was developed as hangover-relieving elixir and its effects on alcoholic metabolism have been investigated. The enzymatic activity of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) of GS-SP was shown higher than those of single treatment with PR, PL, SC, and the positive control group (YM-808). The survival rate of mouse liver cell line NCTC clone 1469 in the presence of acetaldehyde was 30.6, 22.2, and 8.7% at the GS-SP dosage level of 50, 100, and 200 μg/mL respectively. Different concentrations of 50, 100 and 200 mg/kg of GS-SP showed efficient activity for ADH and ALDH than YM-808 in rat fed with 25% ethanol. The levels of blood alcohol and acetaldehyde after oral administration of 200 mg/kg of GS-SP showed efficient activity of 11.7% and 37% than those of YM-808. These results have been supported to the potential for GS-SP to serve as an excellent potential in providing hangover relief and liver protection.
Keywords – *Pueraiae lobata*, *Sorbus commixta*, Alcohol dehydrogenase, Acetaldehyde dehydrogenase, Hangover

Introduction

The positive effects of alcohol consumption are increase in appetite, release of stress and relaxation. However, the abuse of alcohol and chronic consumption can cause the adverse health effects. Most of the absorbed alcohol in stomach and small intestine is oxidized by alcohol dehydrogenase (ADH) to acetaldehyde which is then oxidized to acetic acid by aldehyde dehydrogenase (ALDH) in the liver.[1] Acetaldehyde, the primary metabolite, is the main cause of acute alcohol-hangover symptoms which is connected to deterioration of work quality.[2] Also, fatty liver can result from excessive alcohol consumption, and continuous alcohol absorption can lead to liver cirrhosis. As a result, various products have been released that decrease the accumulation of fat in the liver, prevent liver-damage, and alleviate alcohol-related hangovers.[3] However, the mechanism of these products has not been revealed and scientific research into this is required.

Pueratia folium (PF), young shoots of *Pueraria lobata* Ohwi (*P. lobate)* has levels of puerarin, daidzein and *β*-sitosterol similar to that of Pueraria radix (PR). Major effects of flavonoid extracted from PR are increasing blood flow to the brain and coronary artery, spasmolytic effect, fever reduction, and muscle relaxation.[4] *Sorbus commixta* (SC) were used as folk medicine for coughs, neuralgia, asthma, and roborant.[5] Its major components including catechin-7-*O*-*β*-D-xylopyranoside, catechin-7-*O*-*β*-D-apiofuranoside, prunasin, lupenone, and lupeol are reported to decrease alcohol concentration and inflammation.[6-7] The hangover agent on the market are generally having a blood alcohol resolution and gastric protection function.

In this study, we determined ideal mixture proportioning using PF, PR, and SC, which are known to be excellent in preventing liver damage and oxidative stress[4,7] and investigated its biological activities to develop functional food materials for hangover relief and liver protection.

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Experimental

Material – Pueraiae Radix (PR), Pueratia Folium (PF) and *Sorbus commixta* (SC*)* were purchased from local market (Geumsan, Korea). The samples were deposited in the International Ginseng and Herb Research Institute [(No.; GS201203 (PR)], GS201204 (PF), GS201205 (SC)). Yeo-Myung 808 (YM-808) was purchased from Glami (Glami co., Korea). Alcohol dehydrogenase (Sigma A7011-

30KU), Aldehyde dehydrogenase (Sigma 82884) and Nicotinamide adenine dinucleotide (NAD^+) (Sigma 43407) were purchased from Sigma (Sigma chemical. co., USA).

Extraction and mixture – Each sample powder (50 g) was extracted with 80% ethyl alcohol at 80 °C for 5h. This procedure was conducted at least 3 times before being filtered and concentrated using vacuum evaporator. Positive control (YM-808) concentrated using vacuum evaporator. The extracted samples were diluted in distilled water and mixed with the enzymes ADH and ALDH after their activity was measured. The optimized mixture ratio (*w/w/w*) was set to Pueraiae Radix (PR), Pueratia Folium (PF), *Sorbus commixta* (SC) mixture (GS-SP, PR: PL: SC = 1: 2: 0.5).

Alcohol dehydrogenase (ADH) enzyme activity – ADH activity was measured by following Reacker's method[8], using spectrophotometer set at 340 nm to measure the absorption of NADH. Thus, a total of 1.8 mL solution in test tube included 0.1 mL alcohol, 0.5 mL NAD aqueous solution (2 mg/mL), 0.1 mL of the sample at the various concentrations, and 0.01 M glycine-NaOH buffer solution (pH 8.8) was reacted in 25 °C constant temperature water bath for 10 min and then ADH (18 units/mL) 0.25 mL was added before the absorbance was measured at 340 nm. The control group had 0.25 mL of 0.01 M glycine-NaOH buffer solution (pH 8.8) in place of ADH. ADH activity was estimated by comparing the measured maximum absorbance upon completion of the reaction with the absorbance of the control group and calculating the relative percentage activity.

Aldehyde dehydrogenase (ALDH) enzyme activity – ALDH activity was measured using modified Tottmar's method,[9] measuring the change in absorbance at 340 nm to determine the change in NADH levels. To evaluate the activity, 0.1 mL ALDH (1 unit/mL) and 0.1 mL of each concentration level of sample were added to test tube containing 50 mM sodium pyrophosphate buffer solution (pH 8.8), 0.5 mM NAD, 0.1 mM pyrazole, 5 mM acetaldehyde reaction solution and placed in a water bath at 25 °C for 10 min before measuring absorbance at 340 nm.

Measurement of cell viability – NCTC clone 1469 cells (5×10^4) were cultured in 96-well plates for 24 h at 37 °C. After the incubation, the GS-SP extracts were treated to each well according to the concentration, and then the cells were incubated at 37 °C for 30 min. After the treatment of the extracts, 1 μM acetaldehyde was applied to each well and then the cells were re-incubated at 37 °C for 24 h. And then, 50 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT, 1 mg/ml) was treated to each well. The supernatant was removed after 4 h, and then 100 μL of DMSO was treated to each well. The observance was measures with a micro plate reader at 570 nm.

Animal treatments – Sprague-Dawley (SD) male rats 4-week-old, 90 ± 10 g weight were used in this experiment and were well adapted to the laboratory conditions for 1 week before starting the experimentation. To determine the effects of GS-SP extract on hangovers, experiments for measuring the blood alcohol and acetaldehyde levels were measured at time lapsed condition after alcohol consumption. Animal experiment was conducted with distilled water administered to the control group and GS-SP mixture to the test group at 50, 100, 200 mg/kg of body weight. Positive control group used the commercially available hangover relief beverage (YM-808). Each group had a single oral administration of the sample, and after 1 h, 25% ethanol (15 mg/kg of body weight) was administered. The blood levels of ADH, ALDH, acetaldehyde, and alcohol were measured after 2, 4, and 8 h, respectively. The temperature and humidity of animal room were maintained at 23 ± 1 °C and 50 ± 5% respectively, light and dark periods were set at 12 h intervals. During the raising, water and food were freely available, without interruption.

Ethanol and acetaldehyde concentration in serum – Blood was collected in time lapse period after alcohol administration and left at room temperature for 30 min before centrifugation at 1500 rpm for 15 min and the supernatant was used. Ethanol and acetaldehyde concentration of serum were evaluated by ethanol estimation kit (Roche Co., Darmstadt, Germany) and acetaldehyde estimation kit (R-Biopharm, Germany). Ethanol is decomposed by ADH to acetaldehyde and oxidized by ALDH to acetate, and in this procedure NAD^+ is converted to NADH. Thus the concentration of NADH was determined by measuring absorbance at 340 nm.

Statistical analysis – The results was expressed as mean ± S.E. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test (SPSS VER. 21; SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p < 0.05$.

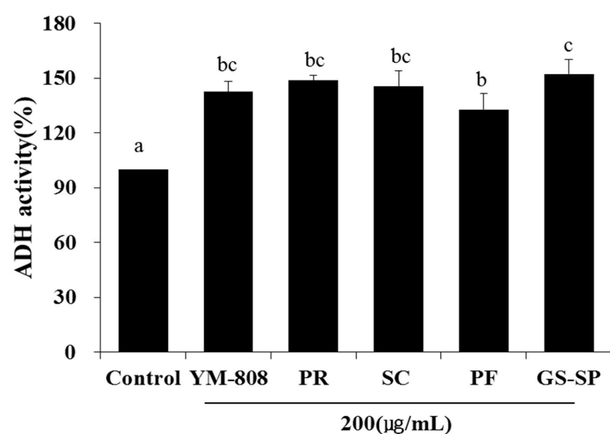
Results

Extraction yield rate – Sample extraction with 80% of ethyl alcohol at 80 °C for 5 h was conducted 3 times. PR showed the highest yield at 23.1%, while PF showed a slightly similar yield to PR at 22.4%. SC resulted in the lowest yield at 20.2%.

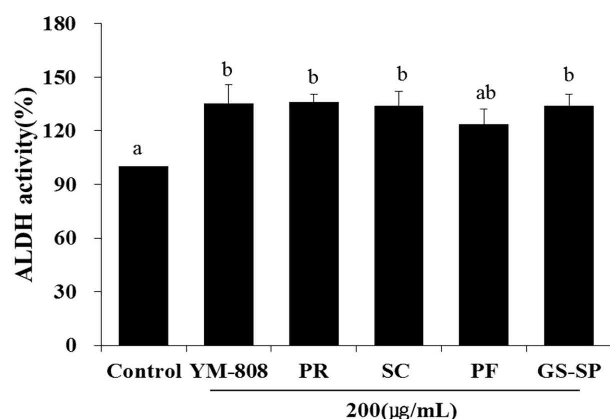
Table 1. Yield of fractions extracted from medicinal plant

Name	Yield (%, *w/w*)
Pueraria rdix (PR)	23.1
Pueraria flos (PF)	22.4
Sorbus commixta (SC)	20.2

Yield (%) = weight of solid extract / weight of dry sample × 100



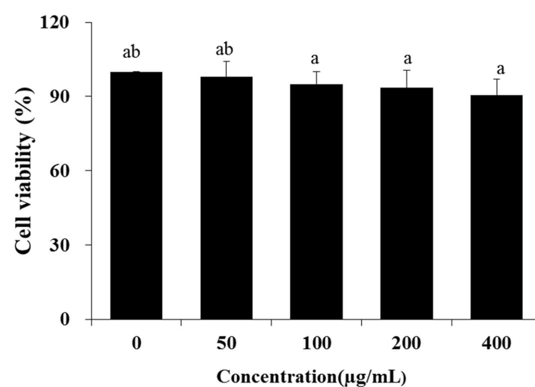
(A)



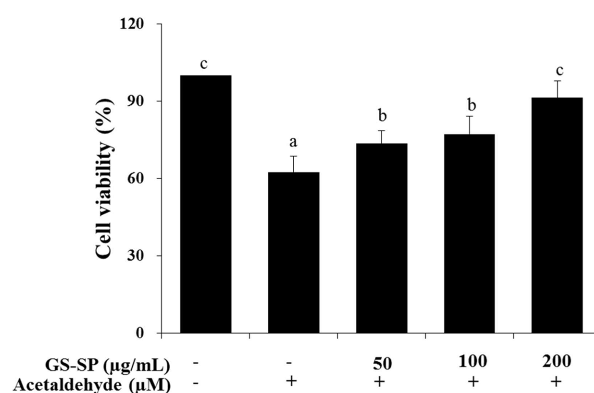
(B)

Fig. 1. Activities of extracts of various medicinal plant extracts on alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Values are mean ± SE (n = 3). Same letters (a-c) are not significantly different at $p < 0.05$ by Tukey's multiple range test after one-way analysis of variance (ANOVA).

ADH and ALDH enzyme activity – ADH and ALDH enzymes are critical for the process of alcohol decomposition. The enzyme activity of ADH and ALDH in the control group was set to 100% and the other groups were measured in comparison to them (Fig. 1). ADH enzyme activity appeared as 155.3, 150.1, 146.3, and 128.7% for GS-SP, PR, SC, and PF, respectively, when treated with 200 μg/mL, while YM-808 used as positive control group gave 141.4%. ALDH enzyme activity appeared as 135.3, 133.5, 128.6, 126.2, and 133.4% for GS-SP, PR, SC, PF and YM-808, respectively. ADH and ALDH enzyme activity seemed to indicate a synergistic effect for the activity of extract-mixture was significantly higher than that of single extract.



(A)



(B)

Fig. 2. Cytotoxicity (A) and inhibitory effect of GS-SP on cell death induced by acetaldehyde (B). The viability of NCTC clone 1469 cells on the treatment of acetaldehyde was evaluated by a MTT assay. Cell viability means the relative values of the negative control (untreated cells).Values are mean ± SE (n = 3). Same letters (a-c) are not significantly different at $p < 0.05$ by Tukey's multiple range test after one-way analysis of variance (ANOVA).

Inhibitory effect of GS-SP on cell damage induced by acetaldehyde – The cell viability was determined by MTT assay. NCTC clone 1469 cells were treated with various concentrations of GS-SP for 24 h. As shown in Fig. 2A, GS-SP did not exhibit cytotoxicity at the range of 50 - 400 μg/mL against cells. This dose-range was used for the treatment of GS-SP in the further experiments. The effect of the GS-SP on the acetaldehyde cell damage was evaluated by MTT assay (Fig. 2B). In MTT the cells treated with acetaldehyde alone induced the cell death by approximately 40% compared with the untreated cells (control), while the addition of the GS-SP in presence of acetaldehyde inhibited the oxidative cell death. GS-SP at

Table 2. Measurement of alcohol dehydrogenase activity

Time (hr)	Alcohol	GS-SP (mg/kg)			YM-808 (mg/kg)		
		50	100	200	50	100	200
2	61.7 ± 2.1[a]	63.4 ± 1.3[ab]	65.5 ± 2.3[d]	92.2 ± 2.5[b]	65.0 ± 2.0[ab]	67.0 ± 1.5[cd]	83.9 ± 2.3[d]
4	68.6 ± 1.5[b]	69.0 ± 1.5[d]	72.3 ± 0.6[ab]	93.7 ± 3.6[ab]	67.0 ± 1.8[b]	69.0 ± 2.1[a]	87.0 ± 2.6[bc]
8	92.1 ± 0.9[a]	95.8 ± 0.6[a]	98.6 ± 0.5[a]	110.0 ± 0.9[a]	91.8 ± 2.5[bc]	91.3 ± 1.8[cd]	98.3 ± 2.0[cd]

Values are mean ± SE (n = 3).
Same letters (a-d) are not significantly different at $p < 0.05$ by Tukey's multiple range test after one-way analysis of variance (ANOVA).

Table 3. Measurement of Aldehyde dehydrogenase activity

Time (hr)	Alcohol	GS-SP (mg/kg)			YM-808 (mg/kg)		
		50	100	200	50	100	200
2	72.0 ± 2.4[a]	82.0 ± 0.8[c]	86.0 ± 2.3[d]	92.2 ± 2.0[b]	75.6 ± 2.4[a]	85.0 ± 2.0[d]	89.0 ± 1.6[cd]
4	75.0 ± 1.2[a]	83.0 ± 1.5[c]	88.0 ± 1.6[d]	93.7 ± 1.9[b]	87.3 ± 1.4[b]	87.0 ± 3.6[c]	93.0 ± 2.0[d]
8	85.0 ± 1.6[ab]	95.8 ± 2.0[d]	98.0 ± 2.4[bc]	105.0 ± 0.8[a]	91.8 ± 1.0[d]	98.0 ± 2.4[c]	98.3 ± 1.4[c]

Values are mean ± SE (n = 3).
Same letters (a-d) are not significantly different at $p < 0.05$ by Tukey's multiple range test after one-way analysis of variance (ANOVA).

different concentrations of 50, 100, 200 and 400 µg/mL showed decrease in cell death rates of 30.6, 22.2, and 8.7%, respectively. Thus, GS-SP has been concluded to inhibit cell death by decomposing acetaldehyde levels within cells.

Effects of GS-SP on serum ADH and ALDH activities – The relative activity of alcohol metabolizing enzymes were compared with the control group, whose enzyme activity was set to be 100%, as shown in Tables 3 and 4. As both the experimental and positive control group showed a substantial reduction in blood alcohol level over the control group, both ADH and ALDH activity has played a vital role after alcohol consumption. Blood was drawn from the heart at 2, 4 and 8 h after alcohol was administered to determine the activity levels of alcohol metabolizing enzymes (Table 2, Table 3). While the GS-SP and YM-808 showed low enzyme activity at 50 and 100 mg/kg, but the both ADH and ALDH activity of GS-SP was greater than those of YM-808 at the dose of 200 mg/kg. In comparison to the control group, experimental group showed approximately 30% greater enzyme activity compared to the control group between 2 h and 4 h. However, after 8 h, the alcohol metabolizing enzyme function in the control group returned to normal.

Effects of GS-SP on serum alcohol concentrations – In order to determine the effects of GS-SP on relieving hangovers, the blood alcohol content was investigated. Measurements were made from blood withdrawn at 2, 4 and 8 h after alcohol was administered. As documented in past studies where blood alcohol concentration is reported to be highest 1 - 2 h after alcohol consumption, blood withdrawn at 2 h had the highest alcohol concentration. The dose of 200 mg/kg of GS-SP and YM-808 were both administered orally 1 h before 25% ethanol (15 mL/kg) consumption, and both showed similar blood alcohol concentration after 2 h. However, GS-SP at 4 h after alcohol consumption showed approximately 20% greater degradation of alcohol over YM-808. At 8 h after consumption, GS-SP and YM-808 resulted in 0.082 ± 0.08 and 0.088 ± 0.005 g/L, respectively, indicating that alcohol concentration reduction normalizes after a certain period of time (Fig. 3). After the 4 h point, the natural alcohol degradation pathway took over and both groups

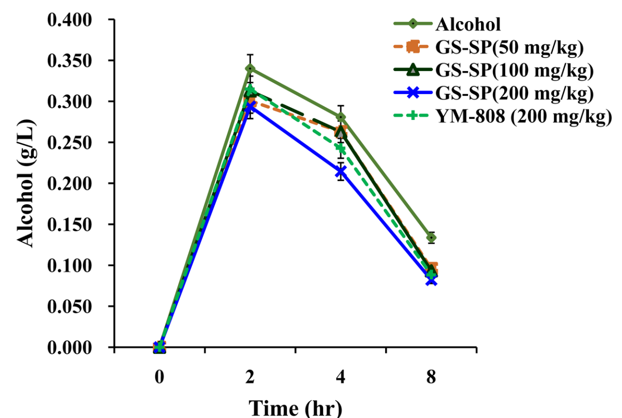


Fig. 3. Effects of GS-SP time dependent changes of blood alcohol concentration after administration of ethanol in drunken rats. Values are mean ± SE (n = 5). GS-SP : *Sorbus commixta* + *Pueraria lobata.*

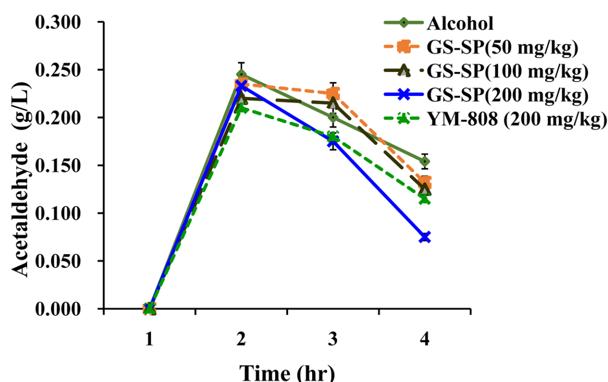


Fig. 4. Effects of GS-SP time dependent changes of blood acetaldehyde concentration after administration of ethanol in drunken rats.Values are mean ± SE (n = 5). GS-SP: *Sorbus commixta* + *Pueraria lobata.*

ceased to show a substantial difference.

Effects of GS-SP on serum acetaldehyde concentrations – The concentration of acetaldehyde in the control group showed the greatest blood alcohol concentration after 2 h at 0.245 ± 0.012 g/L. Although the acetaldehyde concentration was not noticeably different at 2 h, the experimental and positive control group showed a decrease in concentration with further passage of time in a concentration-dependent manner. Both GS-SP and YM-808 showed similar activity at 50 and 100 mg/kg. However, GS-SP resulted in a 37% greater activity which could be increase than YM-808, when concentration was increased to 200 mg/kg (Fig. 4). Therefore, GS-SP is more active at reducing acetaldehyde levels than YM-808, showing potentiality as hangover relief beverage.

Discussion

Alcohol consumption in small amounts helps to alleviate stress, serving to aid in social life and providing enjoyment. However, alcoholism by excessive or chronic alcohol consumption is a serious problem throughout the world. The development of alcoholism remedies have medical, social and economical significance.[10] Alcohol is mostly absorbed in the stomach and small intestine before moving on to the liver to be metabolized. Approximately 1/3 of the absorbed alcohol is metabolized by alcohol dehydrogenase (ADH) in the stomach, minimizing alcohol absorption. Approximately 90% of the alcohol reaching the liver is oxidized to acetaldehyde by ADH before being oxidized to acetic acid by aldehyde dehydrogenase (ALDH).[11] The remaining 10% is metabolized to acetaldehyde by the enzyme catalase. Acetaldehyde, the primary metabolite of alcohol, is a highly active compound that is able to react with various other compounds, thus causing liver cytotoxicity, necrosis, alteration of microvessel structure, disruption to structure and function of liver mitochondria, and liver damage via oxidation of lipids.[12,13] Acetaldehyde, the main cause of hangovers, has a high affinity for cysteine and glutathione in the liver microsome, which can cause disruption in lipids, causing fatty liver and even necrosis among other liver complications.[14] Also, acetaldehyde can reach the brain where it is converted to harmful compounds, potentially causing increase in heart rate, sweating, telangiectasia, nausea, and vomiting. With the economic growth and increase in average lifespan, modern illnesses and an aging society is altering our perception of quality of life. With this change, greater interest in plant based natural products with antibacterial, antioxidant, anticancer and immune-strengthening qualities have gained much attention. Consumption of healthy functional foods composed of natural products is consequently on the rise and alcohol-related hangover relief beverages are part of this, holding a 100 million US dollar market. As new hangover alleviating beverages come out to the market, various researches into the mechanisms of these products are underway, but without conclusive results, more scientific verification is required.[15-18]

In our research based on these considerations, we screened folk application of traditional hangover remedies and clinical application of herbal complex and patent medicines for alcoholism treatment. We determined the effects of PR, PF and SC extracts on the enzyme activity of ADH and ALDH, leading to the production of a combination of these three extracts resulted to provide the maximum synergistic effect. ADH activity showed to be 155.3, 150.1, 146.3, and 128.7% in GS-SP, PR, SC and PR, respectively, with an activity of 141.4% with the positive control group YM-808. Analysis of ALDH activity showed 135.5, 133.5, 128.6, and 126.2% in GS-SP, PR, SC and PR, respectively, with an activity of 133.4% with the positive control YM-808. ADH and ALDH activity increased 5.3 and 2.3%, respectively, when treated with GS-SP alone. Normal liver cells (NCTC clone 1469) were treated with acetaldehyde to induce cell death, after which cell survival rate was measured to be 46.6% with no further treatment. Those treated with GS-SP at concentrations of 50, 100, and 200 µg/mL showed decrease in cell death rates of 30.6, 22.2, and 8.7%, respectively. Therefore, GS-SP was determined to decompose acetaldehyde in cells, thus reducing cell death rate. The relative effects of different doses of GS-SP on the activity of alcohol metabolizing enzymes after oral delivery were measured against the blank control group,

which was set to be 100%. Blood was withdrawn from the heart at different intervals of 2, 4, and 8 h, after administration of alcohol in order to determine the activity level of alcohol metabolizing enzymes. Enzyme activity in both the experimental GS-SP and positive control groups YM-808, showed low activity at the dose of 50 and 100 mg/kg. However, at the dose of 200 mg/kg, enzyme activity was 11.7% higher in the experimental group than the positive control group. The blood acetaldehyde levels in the alcohol treated group 2 h after administration, showed the highest concentration at 0.25 g/L. Although acetaldehyde levels did not decrease after 2 h, the levels in the other groups would decrease in a concentration-dependent manner with further passage of time. The time-dependent decrease in acetaldehyde levels for GS-SP and YM-808 were identical at dose of 50 and 100 mg/kg. However, at the dose of 200 mg/kg, GS-SP showed approximately 37% greater activity than YM-808.

In conclusion to this study, GS-SP was shown to increase enzymatic activity of ADH and ALDH, ultimately reducing blood alcohol and acetaldehyde levels in rat. This in turn consequently leads to hangover relief and prevention of alcoholic liver- damage.

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