Protective effect of heat-treated cucumber (*Cucumis sativus L.*) juice on alcohol detoxification in experimental rats

Vivek K Bajpai[1,$], Na-Hyung Kim[2,$], Ji-Eun Kim[3], Kangmin Kim[4,*] and Sun Chul Kang[3,*]

[1]Department of Applied Microbiology and Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk South Korea
[2]Department of Oriental Pharmacy, Wonkwang University, Iksan-city, Jeonbuk, Republic of Korea
[3]Department of Biotechnology, College of Engineering, Daegu University, Gyeongsan, Gyeongbuk, South Korea
[4]Division of Biotechnology, College of Environmental and Bioresource Sciences, Chonbuk National University, Gobong-ro, Iksan-si, Joellabuk-do (Jeonbuk), Korea

Abstract: In this study, heat-treated cucumber juice was assessed for its protective effect on blood alcohol levels and hepatic alcohol metabolic enzyme system in experimental rats. Initially, during detoxification of alcohol, all groups were orally dosed to 22% alcohol (6 ml/kg body weight) along with different concentrations of heat-treated cucumber juice (10, 100 and 500 mg/kg) and commercial goods for hangover-removal on sale (2 ml/kg). Cucumber juice was dosed before 30 min, and simultaneously after 30 min of alcohol administration, and its hepatoprotective effect on blood alcohol levels and hepatic alcohol metabolic enzyme system in experimental rats was evaluated. As a result, after 7 h, remarkable reduction was found in the blood alcohol levels for all concentrations of cucumber juice treatment. Treatment with cucumber juice resulted in increasing dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) enzymatic activities in rat liver at 9 h after alcohol administration thereby stimulated blood alcohol metabolism as compared with control group. The effect of heat-treated cucumber juice on alcohol detoxification was observed only in the rats treated before 30 min from alcohol administration. These findings indicate that heat-treated cucumber juice has significant protective effect on alcohol detoxification in experimental rats, suggesting its usefulness in the treatment of liver injury caused by alcohol consumption.

Keywords: *Cucumis sativus*, cucumber juice, alcohol detoxification, ADH, ALDH.

INTRODUCTION

A hangover occurs several hours after alcohol consumption with exception at about zero level of blood alcohol (Verster *et al.*, 2010). Social drinkers complain of various discomforting conditions related to a hangover on the day after drinking alcohol. Penning and McKinney (2012) reported that 75% of people who drink alcohol experience a hangover once or more. Consumption of alcohol after its complete metabolism with ability to cause body dysfunction called hangover, which includes fatigue, headache, poor sensing ability, fatigue and diarrhea (Wiese *et al.*, 2000). Initially, absorption of alcohol occurs at gastrointestinal tract from where it is circulated rapidly with a uniform distribution to other vital organs in body (Slustke *et al.*, 2003). Further, enzymatic oxidization of absorbed alcohol caused by alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) results in the formation of acetaldehyde and acetate in liver (Hong *et al.,* 2015). The ADH pathway is a major pathway for alcohol metabolism in the liver. Recent reports have shown positive association between ADH genotype and alcoholic liver injury or even alcoholism (Quertemont, 2004).

Modern medicinal research has emerged with a number of novel drugs of pharmaceutical significance. Complications of liver damage have not got curable therapies to some extent even by a single or combined treatment strategies. Due to versatile functional role of live in body, it has been found very susceptible and easily got damaged by alcohol consumption. Moreover, other liver injuries caused by major chemicals CCl_4 and Pb, including antibiotics have significant adverse effect on proper liver functioning (Wiese *et al.*, 2000). Currently, most of the commercially hepatoprotective agents are found as a poly-herbal formulation which contain enough number of herbal extracts with enhanced amount of antioxidant compounds (Rajashekar *et al.*, 2012).

Moreover, liver plays a vital role in human body as an important activist of biochemical reactions. Liver plays a crucial role in the detoxification process of body and shows enormous ability to synthesize biologically important biomolecules (Anantha *et al.*, 2012).

Cucumber has gained plenty of research subjects being a processed food stuffs and there is no scientific evidence that cucumber has been used as a functional food with hepatoprotective effect. Moreover, the fresh cucumber is nutritiously a very good source of vitamins, minerals and dietary fiber. Due to severe adversary effects of chemical liver-protecting agents, there is an urgent need to explore

**Corresponding authors:* e-mails: sckang@daegu.ac.kr/activase@jbnu.ac.kr
[$]Equally contributed.

safe and natural hepatoprotective agents from plant origin. Thermal pasteurization can prevent spoiling and diminish growth of harmful pathogens as well as extends shelf-life of processed food, hence, in this study, heat-treated cucumber juice was tested for evaluating its protective effect on blood alcohol levels and hepatic alcohol metabolic enzyme system in experimental rats.

MATERIALS AND METHODS

Sample preparation

Cucumbers (*Cucumis sativus L.*) were purchased from an agricultural market in Gun-Wi, Gyeongbuk, Republic of Korea, and stored at -20°C until further processing. After thawing for 2-3 h at 4°C, the cucumbers were subjected to crush and filtered with Whatman No. 2 filter paper to gain raw cucumber juice. Further, this cucumber juice was subjected to heat treatment in a water bath for 40 min at 80°C, followed by re-filtration, and freeze-drying, and stocked at -20°C until used.

Experimental animals

Male Sprague-Dawley rats used in this study were obtained from Orient Co. Ltd., Republic of Korea. Animal were fed with standard chow diet, and tap-water. Animals were kept under standard conditions (temperature; 24±1°C, relative humidity; 55±3% and 12 h light/dark cycle). All rats were allowed to acclimatize for 1 week prior to experimentation. National ethical approval (Regd. No. 0052008-DGU) was obtained for experimental animals on "Animal Care and Use" by the ethical committee of Daegu University, Gyeongsan, Korea.

Detoxification of alcohol

Experimental rats of 120±10 g weight were used in this study. A previously reported method was adopted for the detoxification of alcohol (Hong *et al*., 2015). All groups were orally dosed to 22% alcohol (6 ml/kg body weight) along with different concentrations of heat-treated cucumber juice (Low-dose-10 mg/kg; Medium-dose-100 mg/kg; High-dose-500 mg/kg) and commercial goods for hangover-removal on sale (2 ml/kg). Treatments to the rats were given in the following three different delivery manners: (1) cucumber juice diet before 30 min to alcohol administration, (2) simultaneous cucumber juice diet and alcohol administration (3) cucumber juice diet after 30 min to alcohol administration. Based on dietary categories, rats were sub-divided into the thirteen groups (n=10) including no treatment, as followings: Group 1: Normal diet as a control (C); Group 2: Alcohol treatment with normal diet and saline supplement as a negative control (NC).

Group 3: 10 mg/kg cucumber juice with normal diet before alcohol administration (Pre-10CA); Group 4: 100 mg/kg cucumber juice with normal diet before alcohol administration (Pre-100CA); Group 5: 500 mg/kg cucumber juice with normal diet before alcohol administration (Pre-500CA) and Group 6: 2 ml/kg hangover-removal goods with normal diet before alcohol administration as a positive control (Pre-PC).

Group 7: 10 mg/kg cucumber juice with normal diet and simultaneously alcohol administration (Sim-10AC); Group 8: 100 mg/kg cucumber juice with normal diet simultaneously alcohol administration (Sim-100AC) and Group 9: 500 mg/kg cucumber juice with normal diet simultaneously alcohol administration (Sim-500AC)

Group 10: 10 mg/kg cucumber juice with normal diet after alcohol administration (Post-10AC); Group 11: 100 mg/kg cucumber juice with normal diet after alcohol administration (Post-100AC); Group 12: 500 mg/kg cucumber juice with normal diet after alcohol administration (Post-500AC) and Group 13: 2 ml/kg hangover-removal goods with normal diet after alcohol administration as a positive control (Post-PC).

Measurement of blood alcohol level

Blood was collected from the heart of rats fed with alcohol after 1, 3, 5 and 7 h anesthetized with ethyl ether in a heparin treated Eppendorf tube. According to the modified method of Gutmann (1974), 0.1 ml of blood sample and 0.8 ml of 0.33 N perchloric acid was mixed followed by centrifugation (3,000xg for 5 min), and the supernatant was used for the analysis of blood alcohol levels. For the analysis, 33 µl of the supernatant of blood sample was added to reagent [0.9 ml of 48 mM glycine buffer, 33 µl of 24 mM β-NAD (β-nicotinamide adenine dinucleotide), and 38 µl ADH (yeast); 36 U/ml of total reagent] and the mixture was reacted at 25ºC for 70 min. Subsequently, this mixture was read spectrophotometrically at 340 nm. Amount of alcohol concentration was measured using standard absorbance obtained from the standard curve.

Determination of enzyme levels in serum

Enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using commercial kits with auto dry chemistry-analyzer (Spotchem™ SP-4410®, Kyoto Daiichi kagaku Co., Ltd. Japan).

Measurement of hepatic enzyme activities related to alcohol metabolism

Alcohol-treated animals were sacrificed after 9 h under ethyl ether anesthesia. Livers samples were cut, rinsed with PBS. After trimming away connective tissues using freeze liquid nitrogen, samples were preserved at -70°C for further analysis. Liver samples were subjected to homogenization using a sucrose solution (0.25 M), followed by centrifugation (6000×g; 10 min) for the removal of cell debris and collection of cell free supernatant. Further, supernatant was ultracentrifuged

(10000×g; 20 min) to get rid of mitochondrial pellet, and fraction of cytosol was collected from ultracentrifuged supernatant. The cytosol fraction was used for ADH activity assay, and the mitochondrial fraction was used for ALDH activity assay, respectively. ADH activity was assayed after 10 min reaction at 25°C using a UV Spectrometer at 340 nm and measured in 1-mL cuvettes containing 1 mM NAD, 48 mM glycine-NaOH buffer (pH 9.6) with 3 mM EtOH, and 50 µl of cytosol. ALDH activity was also assayed after 2 min reaction at 25°C using UV spectrometer at 340 nm. Reaction solution (1.0 ml) consisted of 1 mM NAD, 50mM sodium phosphate buffer (pH 7.4) with 5 mM propanol and 50 µl of mitochondrial fraction. Activities were expressed as the rate of NADH formation µmol/min/mg protein and the quantity of protein was determined by the Bradford assay (Bradford, 1976).

STATISTICAL ANALYSIS

Mean differences were obtained and data were analyzed using one-way analysis of variance (ANOVA) at a significant level of $p<0.05$.

RESULTS

Effect of heat-treated cucumber juice on blood alcohol levels

Blood alcohol levels from alcohol-administered rats were measured at 1, 3, 5 and 7 h after oral administration of 22% alcohol and cucumber juice (fig. 1A, 1B, 1C). As shown in fig. 1A, when rats were treated with various cucumber juice concentrations before 30 min of alcohol administration, no significant effect was observed after 1, 3 and 5 h as compared to Pre-PC and AS. However, after 7 h, remarkable reduction was found in the blood alcohol levels for all concentrations of cucumber juice treatment. When alcohol was simultaneously given with cucumber juice (Sim-10AC, Sim-100AC, Sim-500AC), all groups found to be similar in regard of the blood alcohol levels (fig. 1B), confirming that simultaneous administration of cucumber juice did not reveal any effect on hangover-removal. Further, when measured the blood alcohol levels after treatments with various cucumber juice concentrations after 30 min of alcohol administration, there was no significant effect on the reduction of blood alcohol levels at 1, 3 and 5 h as compared to Post-AC and AS. Significant reduction was observed after only 7 h after 30 min of alcohol administration. However, the treatment of commercial hangover-removal goods significantly reduced of the blood alcohol levels up to 0.01% as compared to AS (fig. 1C).

Activity of marker enzymes related to hepatic damage

Previously ALT, AST and ALP have been used as significant biomarkers for the diagnosis of liver injuries. The effect of heat-treated cucumber juice on alcohol-induced serum enzymes AST, ALT and ALP in rats has been shown in fig. 2A, 2B, 2C. As shown in fig. 2A and 2C, in Pre-500CA treated group, AST and ALP levels in serum were decreased dramatically as compared to AS values, respectively. On the other hand, cucumber juice treatment significantly reduced the ALT levels in Pre-10CA and Pre-500CA as compared to AS, whereas no significant reduction in AST levels was observed in Pre-100CA treated rats (fig. 2B). The results of this assay confirmed that administration of cucumber juice reduced the activities of AST, ALT and ALP in alcohol administered animals indicating its hepatoprotective against liver injury caused by alcohol consumption.

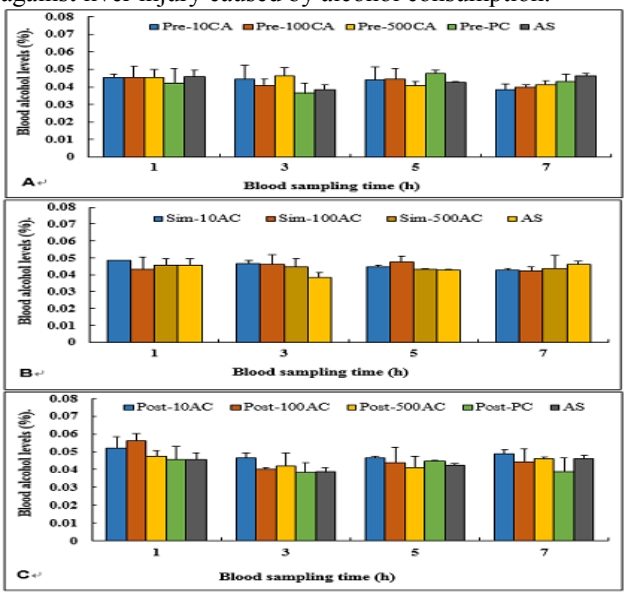

Fig. 1: Effect of heat-treated cucumber juice on blood alcohol levels. (1A) Pre-dosing treatment (before 30 min of alcohol administration) of heat-treated cucumber juice; (1B) Simultaneous treatment of heat-treated cucumber juice; and (1C) Post-dosing treatment (after 30 min of alcohol administration) of heat-treated cucumber juice with the concentration of 10 mg/kg (Pre-10CA), 100 mg/kg (Pre-100CA), 500 mg/kg (Pre-500CA) and commercial goods for hangover-removal (Pre-PC). AS: only alcohol.

Effect of cucumber juice on alcohol oxidizing enzymes ADH and ALDH

ADH and ALDH enzymes are critical for the process of alcohol decomposition. As shown in fig. 3A and 3B, the ADH and ALDH activities for cucumber juice treatment groups after 9 h from alcohol administration were increased as compared to exclusively alcohol-dosed group. Measurement of enzyme activity in 9 h was determined by preliminary experiments. The results of this assay suggested that the heat-treated cucumber juice significantly enhanced the liver ADH and ALDH activities. The results suggested that cucumber juice increased enzymatic activities of ADH and ALDH, thereby stimulated liver metabolism of alcohol in alcohol-

treated rats.

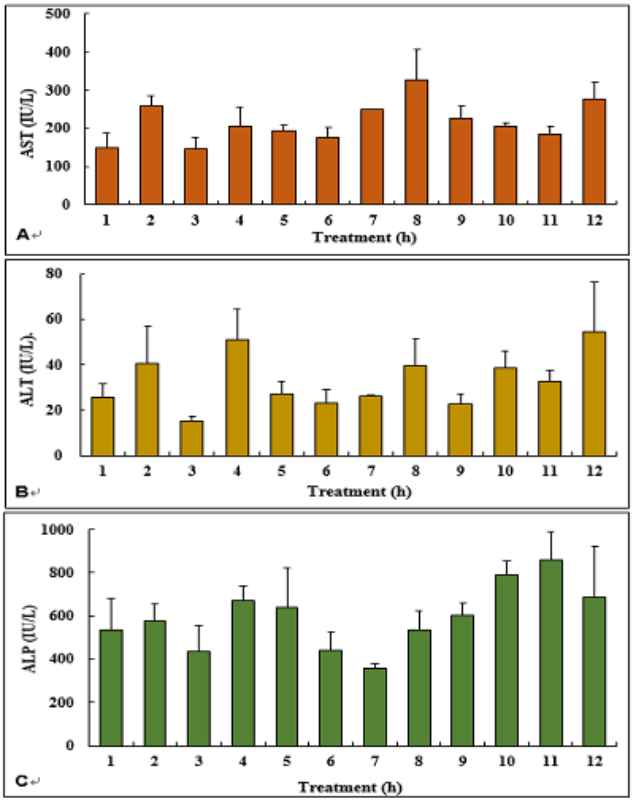

Fig. 2: Effect of heat-treated cucumber juice on the serum marker enzymes. (2A) Serum AST activity in rats; (2B) Serum ALT activity in rats; and (2C) Serum ALP activity in rats treated with various concentrations. 1: Pre-10CA, 2: Pre-100CA, 3: Pre-500CA, 4: Sim-10AC, 5: Sim-100AC, 6: Sim-500AC, 7: Post-10AC, 8: Post-100AC, 9: Post-500AC, 10: Pre-PC, 11: Post-PC, 12: AS

DISCUSSION

This study confirmed that the effect of heat-treated cucumber juice on alcohol detoxification was observed only in the rats treated before 30min of alcohol administration, and the reduced level of alcohol was obtained after only 7 h of alcohol consumption. In a previous study conducted on alcohol administered rats, Lee *et al.* (2013) observed the efficacy of juice of *Pyrus pyrifolia* on hangover severity and reported that the juice of *P. pyrifolia* dramatically reduced average of hangover severity at 15 h after the alcohol consumption, thus suggesting that heat-treated cucumber juice could also be used as an alternative to pear juice for rapid reduction in blood alcohol level.

Increase abnormal activities of AST and ALT are of clinical and toxicological importance, being indicative of tissue damage or disease in rats induced by liver toxicants including alcohol. These toxicants cause tissue damage in the liver, which results in the increment of various hepatic enzymes, such as AST, ALT and ALP (Usunomena *et al.*, 2012). As reported previously, the activities of marker enzymes in liver were found very much susceptible in experimental strategies applied for the determination of liver disorders (Usunomena *et al.*, 2012) and the effect of alcohol removal increased in alcohol-treated rats. When considering hepatic toxicity caused by alcohol, a number of studies have confirmed decrease in WBC count, ALP, hemoglobin, BUN, creatine and glucose, suggesting that such parameters have severe adversary consequences due to consumption of alcohol regardless to intervention of heat-treated cucumber juice as also confirmed previously in case of Korean pear juice (Lee *et al.*, 2013), and such findings were also further supported by others (Nakanishi *et al.*, 2003).

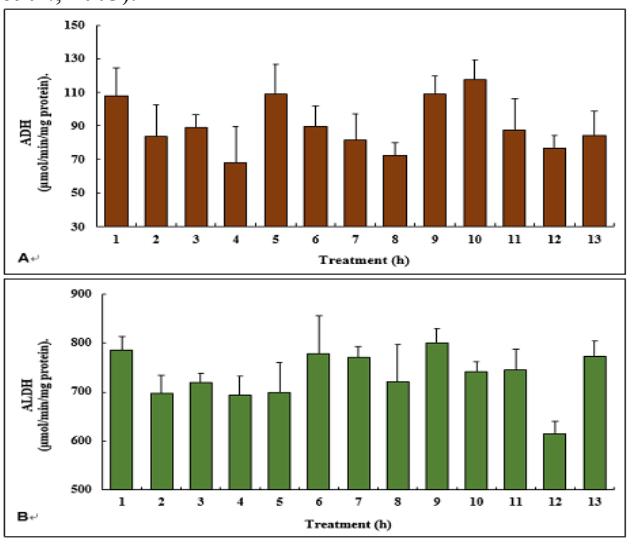

Fig. 3: Effect of heat-treated cucumber juice on alcohol oxidizing enzymes. (3A) Hepatic alcohol dehydrogenase (ADH) activity; and (3B) Hepatic acetaldehyde dehydrogenase (ALDH) activity at 9 h after alcohol intake in alcohol-treated rats. 1: Pre-10CA, 2: Pre-100CA, 3: Pre-500CA, 4: Sim-10AC, 5: Sim-100AC, 6: Sim-500AC, 7: Post-10AC, 8: Post-100AC, 9: Post-500AC, 10: Pre-PC, 11: Post-PC, 12: AC, 13: C (no treatment).

In alcohol-induced hangover, alcohol and alcoholic metabolites such as acetaldehyde work as casual agents of hangover, thus suggesting that activation of liver metabolism could have significant contribution on controlling hangover and associate consequences of hangover. Metabolism of alcohol accomplishes through its oxidation in liver, which is regulated via catabolic process of alcohol metabolizing enzymes ADH and ALDH (Hong *et al.*, 2015). It is known that alcohol metabolizing enzymes ADH and ALDH play a crucial role in the alcoholic metabolism with a known fact that natural plant extracts have significant potential to activate ADH and ALDH (Cho *et al.*, 2005), as also observed in our study. In earlier studies, it was shown that *Pyrus pyrifolia* derived extracts containing antioxidant molecules were able to activate ADH and ALDH

stimulation (Yang and Lee, 2012). *P. calleryana* extract was also able to reduce hangover symptoms through the activation of metabolizing enzymes (Lee *et al.*, 2013). In addition, recently Hong *et al* (2015) reported that the different concentrations of 50, 100 and 200 mg/kg of mixture of *Pueraria lobata* and *Sorbus commixta* extracts showed efficient activities for ADH and ALDH than control group in rats fed with 25% ethanol.

CONCLUSION

In this study, heat-treated cucumber juice significantly reduced blood alcohol levels as well as decreased the serum levels of hepatic damage causing enzymes AST and ALP, and increased the activities of alcohol oxidizing enzymes ADH and ALDH. Based on the above findings it can be concluded that heat-treated cucumber juice could be a natural agent of hepatoprotective efficacy against alcohol-induced hangover. Further, human trials should be performed to confirm its hepatoprotective and anti-hangover ability.

REFERENCES

Anantha KCD, Siva RC and Manohar RA (2012). Hepatoprotective effect of biherbal ethanolic extract against paracetamol-induced hepatic damage in albino rats. *J. Ayur. Integ. Med.,* **3**: 198-203.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72**: 248-254.

Cho MH, Shim SM, Lee SR, Mar W and Kim GH (2005). Effect of *Evodiae fructus* extracts on gene expressions related with alcohol metabolism and antioxidation in ethanol-loaded mice. *Food Chem. Toxicol.,* **43**: 1365-1371.

Gutmann BE (1974). Ethanol determination with alcohol dehydrogenase and NAD. *In*: Methods of enzymatic analysis. Bergmeyer HV, ed. Chemic Int., Deerfield Beach, FL, USA. **3**: 1499-1502,

Hong SC, Yoo JH, Oh MH, Lee H, Park YS, Parthasarathi S, Park JD and Pyo MK (2015). Effect of the mixture of *Pueraria lobata* and *Sorbus commixta* extract on the alcohol-induced hangover in rats. *Nat. Prod. Sci.,* **21**: 98-103.

Lee HS, Isse T, Kawamboto T, Baik HW, Park JY and Yang M (2013). Effect of Korean pear (*Pyruspyrifolia* cv. Shingo) juice on hangover severity following alcohol consumption. *Food Chem. Toxicol.,* **58**: 101-106.

Nakanishi N, Yoshida H, Okamoto M, Matsuo Y, Suzuki K and Tatara K (2003). Association of alcohol consumption with white blood cell count: A study of Japanese male office workers. *J. Int. Med.,* **253**: 367-374.

Penning R, McKinney A and Verster JC (2012). Alcohol hangover symptoms and their contribution to the overall hangover severity. *Alcoh. Alcoh.,* **47**: 248-252.

Quertemont E (2004). Genetic polymorphism in ethanol metabolism: Acetaldehyde contribution to alcohol abuse and alcoholism. *Mol. Psycol.,* **9**: 570-581.

Rajashekar V, Rao EU and Srinivas P (2012). Biological activities and medicinal properties of Gokhru (Pedalium murex L.). *Asian Pac. J. Trop. Biomed.,* **2**: 581-585.

Slustke WS, Piasecki TM and Hunt-Carter EE (2003). Development and initial validation of the Hangover Symptoms Scale: Prevalence and correlates of hangover symptoms in college students. *Alcoh. Clin. Exp. Res.,* **27**: 1442-1450.

Usunomena U, Ademuyiwa AJ, Tinuade OO, Uduenevwo FE, Martin O and Okolie NP (2012). N-nitrosodimethylamine (NDMA), liver function enzymes, renal function parameters and oxidative stress parameters: A Review. *Brit. J. Pharmacol. Toxicol.,* **3**: 165-176.

Verster JC, Stephens R and Penning R (2010). The alcohol hangover research group consensus statement on best practice in alcohol hangover research. *Curr. Drug. Abuse. Rev.,* **3**: 116-126.

Wiese JG, Shlipak MG and Browner WS (2000). The alcohol hangover. *Ann. Int. Med.,* **132**: 897-902.

Yang M and Lee H (2012). Patent # 10-1104427 at Korea: Alcohol-detoxification of the Korean pears and its active compounds, pp.55-61.