Journal of Animal and Veterinary Advances 11 (12): 2070-2074, 2012
ISSN: 1680-5593

Protective Effect of Corn Oligopeptides on Acute Alcoholism in Rodent Models

[1,2]Feng Zhang, [1,2]Jiali Zhang, [1,2]Meihong Xu, [1,2]Xiaoqian Dai, [1,2]Yujie Li,
[1,2]Ye Ding, [1,2]Zhaofeng Zhang and [1,2]Yong Li
[1]Department of Nutrition and Food Hygiene, School of Public Health,
[2]Beijing Key Laboratory of Toxicological Research and Risk Assessment for Food Safety,
Peking University, Xueyuan Road 38, Haidian District, 100191 Beijing, P.R. China

Abstract: The present study aimed to investigate the effects of Corn Oligopeptides (COP) on acute alcoholism in rat models. Female SD rats were randomly assigned to 4 groups per test including control group and 3 COP intervention groups (2.25, 4.5 and 9 g/kg/BW). About 5 g/kg/BW ethanol (8 g/kg/BW for loss of righting reflex test) was given intragastricly 10 min before COP treatment. Ethanol levels in blood, behavioral studies and ADH activity in liver were assayed. Researchers found that COP treatment could promote the alcohol metabolism and correct the movement disorder caused by alcohol consumption to some extent. However, the ADH levels had no changes among different groups. These findings suggest that COP have a protective effect on acute alcoholism in rats.

Key words: Acute alcoholism, corn oligopeptides, ethanol, protective effect, control group

INTRODUCTION

Ethanol exposure remains a major social, economic and health problem worldwide with profound impacts on behaviors (Bayard *et al*., 2004). It is reported that there are nearly two billion alcohol consumers globally and 76.3 million with diagnosable alcohol use disorders (WHO, 2004). Acute alcoholism also, known as alcohol intoxication or drunkenness, occurs when a person reaches a high ethanol level in blood. It may cause a lot of symptoms depending on the quantity of alcohol consumption. Slurred speech and euphoria happens when ethanol concentration in blood is lower and when ethanol level rises, impaired balance and loss of muscle coordination could be observed in severe cases, it can lead to coma or death (Tupala and Tiihonen, 2004).

It is reported that Chinese medicine formulae could be used to relieve alcohol hangover (Haranaka *et al*., 1985). Furthermore, other natural products such as ginseng are also used in folk medicine (Xu *et al*., 2005). It is a brand-new way to find bioactive compounds derived from natural products for the treatment of acute alcoholism. Corn Oligopeptides (COP) which are composed of low molecular weight peptides are obtained by enzymatic hydrolysis from Corn Gluten Meal (CGM), a byproduct of starch industry. The multiple functions of COP have been reported already including inhibiting angiotensin I converting enzyme (Lin *et al*., 2010), alleviating fatigue (Chang, 2004), resisting lipid peroxidation (Xu *et al*., 2002) and facilitating alcohol metabolism (Yamaguchi *et al*., 1997).

Alcohol elimination in the body involves oxidation to acetaldehyde catalyzed by Alcohol Dehydrogenase (ADH) which is followed by further oxidation to acetate (Crabb *et al*., 1987). The changes of alcohol levels are accompanied by behavior disorders such as loss of righting reflex in rodents (Zhang *et al*., 2010; Bahi, 2011). Therefore, ADH is one of the most important enzymes for alcohol metabolism.

Therefore in the present study, the effect of COP on acute alcoholism in rats was investigated by behavioral studies. In addition, the contents of ADH in liver were detected.

MATERIALS AND METHODS

Commercial kits used for determination of ADH in liver were purchased from Jiancheng Institute of Biotechnology (Nanjing, China). Ethanol and other chemicals used were all of analytical grade and purchased from Beijing Chemical Co. (Beijing, China).

Preparation and identification of COP: COP which were prepared from CGM were donated by CF Haishi Biotechnology Co., Ltd. (Beijing, China). Briefly after being ground through a 60 mesh sieve, CGM was

Corresponding Author: Yong Li, Department of Nutrition and Food Hygiene, School of Public Health, Peking University, 100191 Beijing, P.R. China

suspended with distilled water (1:10, w/w) and then hydrolyzed at pH 11.0, 90°C for 1 h. The suspension was neutralized and centrifuged to recover insoluble protein precipitate which was then resuspended and carried out with the same procedure as above to achieve the wet Corn Protein Isolate (CPI). The wet CPI was resuspended to a concentration of 6% (w/w) and performed with a two-step enzymatic hydrolysis. The first step with crude alkaline proteinases was carried out at pH 8.5, 55°C for 3 h. The second step with crude neutral proteinases was done at pH 7.0, 45°C for 2 h. The resultant hydrolysates were centrifuged to remove impurity. After centrifugation, the supernatant was subjected to 10 and 1 kDa MWCO ceramic membranes successively. A procedure of nanofiltration was performed to remove the mineral salt. Then, the purified liquid was condensed by cryoconcentration under a vacuum at 70°C with an evaporation rate of 500 kg h^{-1}. When the concentration was almost 30 Baume degrees, it was decoloured with 12% of active carbon at 75°C for 1 h and then the carbon was removed by filtration. Most of the water was removed by spray drying with a pressure of 20 MPa. COP powder used in the following procedures was thus obtained.

The COP sample contains about 91.61% hydrolysed protein, 5.56 ash, 0.82% carbohydrate, 1.49% water and 0.52% fat. Molecular weight distributions of COP were determined using a HPLC System (LC-20AD, Shimadzu, Kyoto, Japan) on a TSK-gel 2000 SWXL column (7.8×300 mm, Tosoh, Tokyo, Japan) according to the method of Kong *et al*. (2008). HPLC was carried out with the mobile phase (45% acetonitrile with 0.1% TFA, v/v) at a flow rate of 0.5 mL min^{-1}. A molecular weight calibration curve was prepared from the average retention time of the following standards (Sigma, St. Louis, MO, USA): cytochrome C (12.5 kDa), aprotinin (6.5 kDa), bacitracin (1450 Da), tetrapeptide GGYR (451 Da) and tripeptide GGG (189 Da). In addition, total amino acid analysis was carried out with an amino acid analyser (835-50, Hitachi, Tokyo, Japan) according to the method of Yang *et al*. (2007). The results indicated that 96.77% of the peptides in COP were distributed below 1000 Da and the average molecular weight of COP was 363 Da. The average molecular weight of protein amino acids was 137 Da and the mean peptide length was about 2.7. The composition of amino acids is shown in Table 1.

Table 1: Amino acid composition of corn oligopeptides

Amino acid	No. of residues/100 residues
Ala	9.68
Arg	1.79
Asp	5.28
Cys	0.36
Glu	24.21
Gly	1.61
His	1.36
Ile	3.49
Leu	18.27
Lys	0.25
Met	2.51
Phe	5.82
Pro	8.29
Ser	4.83
Thr	2.84
Try	0.26
Tyr	5.51
Val	3.63

Animals: Female SD rats weighing 200-300 g were obtained from the Animal Service of Health Science Center, Peking University and adapted to the vivarium for 1 week before treatment began. The conditions were as follows: in a filter-protected air-conditioned room with controlled temperature (25±28°C), relative air humidity (60±5%) and 12 h light/dark cycles (light on 07:30-19:30 h). Animal treatment and maintenance were carried out strictly in accordance with the Principle of Laboratory Animal Care (NIH publication No.: 85-23, revised 1985) and the guidelines of the Peking University Animal Research Committee.

Experimental procedure: A dose of 50% (v/v) alcohol was given by intragastric administration, 5 g/kg/BW, 10 min before COP treatment. About 8 g/kg/BW alcohol was adopted in the loss of righting reflex test. COP were given at 2.25, 4.5 and 9 g/kg/BW in the intervention groups, respectively defined as low, medium and high dose of COP groups.

Blood ethanol concentration: Blood samples were taken at 30, 60, 120, 180 min following treatment, respectively. 200 μL of blood was taken from angular vein of each subject each time. The samples were immediately placed into individual heparin-treated glass vials with 50 μL tert-butyl alcohol added as internal standard, tightly fastened with a septum sealed lid for further analysis by head space gas chromatography (Shimadzu GC-2010, Japan).

Behavioral study

Loss of righting reflex: Rats were put in a supine position after the treatment. Loss of righting reflex was recorded as the time at which the animal was unable to turn itself. Animals were left in the supine position until recovery of the righting reflex. Recovery of the righting reflex was defined as the time that elapsed until the animal was able to right itself three times in 30 sec. The time to regain the righting reflex was recorded for each animal. The number of rats which lost the righting reflex was also recorded.

Rotarod test: The rotarod apparatus (Hugo Sachs Electronik, Germany) consists of a suspended rod able to run at constant or at accelerating speed. Each subject was placed on a rod (8.9 long and 3.8 cm in diameter) covered with rubber to evaluate rotarod performance at 30 min following treatment. The rotarod was set to 12 r min^{-1} for each trial which continued until the rat had fallen to the padded surface for 3 times or 30 min was reached. The elapsed time, the number of falling rats and the number of rats which were unable to fulfill the test were recorded as the measure of performance on each trial.

Climbing test: At 30 min following treatment, rats were placed onto a metal grid (50 ×100 cm with a mesh size of 1×1 cm) which formed a 60° angle with ground. The elapsed time until the subject had fallen off was recorded for evaluation of muscle tension.

ADH assay: At the end of experiment, rats were sacrificed and livers were dissected on ice. Liver homogenates were prepared and centrifuged (×3000 g) for 10 min. Supernatants were collected for ADH determination. The contents of ADH and protein in the liver homogenate were determined by the detection kits according to the manufacturer's protocols, respectively. ADH activity was expressed in unit per mg protein (u/mgpro), i.e., 1 u/mgpro means that ADH yields 1 nmol product with 1 mg protein per minute at 37°C.

Statistical analysis: Statistical analysis performed with using SPSS for Windows, Version 11.0. Analysis of Variances (ANOVA), χ^2-test or Kruskal-Wallis rank sum test was performed properly $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of COP on blood ethanol concentration: The changes of blood ethanol concentration were shown in Table 2. A steady growing trend was found in control group and low dose of COP group. Both in medium dose and high dose of COP groups, blood ethanol levels reached the peak at 120 min following treatment. With comparison of the control group, the ethanol level in medium dose of COP group at 180 min was lower significantly ($p<0.05$) and at both 120 and 180 min following treatment, the ethanol levels in high dose of COP group decreased significantly ($p<0.05$) compared with control group.

Table 2: Effect of COP on blood alcohol concentration

		Alcohol concentration (mg mL^{-1})[a]			
Groups	n	30 min	60 min	120 min	180 min
Control	6	3.71±1.24	4.01±0.77	4.06±0.51	4.37±1.04
Low COP	6	2.82±1.70	3.54±1.70	3.85±1.01	4.04±0.80
Medium COP	6	2.39±0.66	2.76±1.11	3.04±1.25	2.81±1.21*
High COP	6	2.60±0.60	2.65±0.75	2.73±1.09*	2.52±0.82*

[a]Values are means±SD. *Statistical significance: $p<0.05$, compared with control group. Control group: 5 g/kg/BW ethanol; Low COP: 5 g/kg/BW ethanol+2.25 g/kg/BW COP; Medium COP: 5 g/kg/BW ethanol+4.5 g/kg/BW COP; High COP: 5 g/kg/BW ethanol+9 g/kg/BW COP

Effects of COP on behavioral performance

Loss of righting reflex: The duration of loss of righting reflex produced by ethanol was evaluated and shown in Table 3. With comparison of control group, all doses of COP treatment could significantly decrease the ratio of rats with loss of righting reflex ($p<0.05$). No rats with loss of righting reflex were found in high dose of COP group. Meantime, researchers could found that rats in control group reached the loss of righting reflex earlier than the COP intervention groups and recovered later than the COP groups.

Rotarod test: Rotarod test which measures balance, coordination and motor control was used to evaluate motor performance (Table 4). In high dose of COP group, the elapsed time which was defined as the duration that rats could stayed on the rod was significantly longer than control group ($p<0.05$). However, the low and medium dose of COP groups showed no significance compared with control group.

Climbing test: The muscle tension was evaluated by the climbing test (Table 5). Rats in high dose of COP group

Table 3: Effect of COP on righting reflex

Groups	n	Loss amount	Loss ratio (%)	Loss time (min)	Recovery time (min)
Control	9	7	77.8	94.6	660.7
Low COP	7	1	14.3*	137.0	329.0
edium COP	7	1	14.3*	352.0	345.0
High COP	8	0	0.0*	-	-

*Statistical significance: $p<0.05$, compared with control group. -: No rats with loss of righting reflex were found. Control group: 8 g/kg/BW ethanol; Low COP: 8 g/kg/BW ethanol+2.25 g/kg/BW COP; Medium COP: 8 g/kg/BW ethanol+4.5 g/kg/BW COP; High COP: 8 g/kg/BW ethanol+9 g/kg/BW COP

Table 4: Effect of COP on Rotarod test

		Elapsed time (min)			
Groups	N	Median	Mean rank	Amount lasting >30 min	Amount unable to fulfill
Control	6	1.98	7.50	1	1
Low COP	6	3.07	10.50	1	0
Medium COP	6	3.09	12.58	2	0
High COP	6	12.28	19.42*	0	0

*Statistical significance: $p<0.05$, compared with control group. Control group: 5 g/kg/BW ethanol; Low COP: 5 g/kg/BW ethanol+2.25 g/kg/BW COP; Medium COP: 5 g/kg/BW ethanol+4.5 g/kg/BW COP; High COP: 5 g/kg/BW ethanol+9 g/kg/BW COP

Table 5: Effect of COP on climbing test

Groups	n	Elapsed time (min)[a]
Control	6	8.22±2.11
Low COP	6	9.52±1.41
Medium COP	6	11.73±1.91
High COP	6	16.71±1.70*

[a]Values are means±SD.*Statistical significance: $p<0.05$, compared with control group. Control group: 5 g/kg/BW ethanol; Low COP: 5 g/kg/BW ethanol+2.25 g/kg/BW COP; Medium COP: 5 g/kg/BW ethanol+ 4.5 g/kg/BW COP; High COP: 5 g/kg/BW ethanol+9 g/kg/BW COP

showed a significantly longer duration before falling off than rats in control group ($p<0.05$). But no significance was found in the low and medium dose of COP groups compared with control group.

Effect of COP on ADH levels in liver: The contents of ADH in liver were shown in Table 6. However, no significance was found in COP intervention groups in comparison with control group.

A lot of attention has been recently paid to bioactive compounds from food for multiple physiological activities. It was reported that COP intervention before alcohol consumption could promote the metabolism of alcohol and decrease the ethanol concentration in blood in healthy persons (Yamaguchi *et al*., 1997). However, there are few studies about the effect of COP on acute alcoholism. Therefore, the effect of COP on alcohol intoxication in rats was investigated. The results obtained in the present study showed that COP intervention could alleviate the effect of alcohol consumption on behavioral performance. However, no effects on ADH level in liver were observed.

Behavioral studies are widely used for evaluation of protective effects of compounds on acute alcoholism (Malinowska *et al*., 1999). In the present study, loss of righting reflex, rotarod test and climbing test were adopted. Compared with control group, all COP intervention groups showed a significantly lower ratio of rats lost righting reflex ($p<0.05$) and obviously delay the time at which rats lost reaction and reduce the recovery duration of subjects. Meantime, the high dose of COP group showed longer elapsed time in rotarod test and climbing test than control group. It indicated that to some extent, the COP treatment showed a protective effect on acute alcoholism that could correct the movement disorder and reduce the damage to central nervous system caused by alcohol consumption.

The metabolism of alcohol involves the oxidation of alcohol to aldehyde which is catalyzed by ADH and the oxidation of aldehyde to acetate which is catalyzed by Aldehyde Dehydrogenase (ALDH) (Haseba *et al*., 2003). A decline trend of ethanol concentration in blood could be observed in the medium and high dose of COP groups in comparison with control group. It indicated that COP

Table 6: Effect of COP on ADH activity in liver

Groups	n	ADH (u/mgpro.)[a]
Control	6	22.77±1.05
Low COP	6	19.44±2.70
Medium COP	6	19.04±1.61
High COP	6	20.69±2.70

[a]Values are means±SD. *Statistical significance: $p<0.05$, compared with control group. Control group: 5 g/kg/BW ethanol; Low COP: 5 g/kg/BW ethanol+2.25 g/kg/BW COP; Medium COP: 5 g/kg/BW ethanol+ 4.5 g/kg/BW COP; High COP: 5 g/kg/BW ethanol+9 g/kg/BW COP

treatment could promote the alcohol metabolism. Yamaguchi *et al*. (1996) found that COP administration before alcohol intake in rats could accelerate the elimination rate of alcohol which is in correspondence with this study. However, it was not observed that the ADH activity could be induced by COP treatment. The negative results above could be attributed to the short duration of experiments. It indicated that the protective effect of COP treatment could be attributed to other mechanism needing further studies.

CONCLUSION

The current investigation demonstrated COP showed a protective effect on acute alcoholism. COP may be beneficial as functional foods potentially. However, further studies such as the longer-term design and human trial are needed.

ACKNOWLEDGEMENTS

The researchers gratefully thank the company CF Haishi Biotechnology Ltd. for providing the samples used in this study. There is no conflict of interest. This research was supported (grant 2006BAD27B08) by the Foundation from the Ministry of Science and Technology of the People's Republic of China.

REFERENCES

Bahi, A., 2011. The pre-synaptic metabotropic glutamate receptor 7 mGluR7 is a critical modulator of ethanol sensitivity in mice. Neuroscience, 199: 13-23.

Bayard, M., J. Mcintyre, K.R. Hill, J. Woodside Jr. and J.H. Quillen, 2004. Alcohol withdrawal syndrome. Am. Fam. Physician, 69: 1443-1450.

Chang, Y.Q., 2004. Study on anti-fatigue effect of corn-peptide. Food Sci., 25: 173-178.

Crabb, D.W., W.F. Bosron and T.K. Li, 1987. Ethanol metabolism. Pharmacol. Ther., 34: 59-73.

Haranaka, R., N. Okada, H. Kosoto, S. Ohwada, R. Hasegawa, S. Nakagawa and M. Kobayashi, 1985. Effects of wu-ling-san on alcohol metabolism and alcoholic liver. J. Tradit. Chin. Med., 5: 171-178.

Haseba, T., Y. Tomita, M. Kurosu and Y. Ohno, 2003. Dose and time changes in liver alcohol dehydrogenase (ADH) activity during acute alcohol intoxication involve not only class I but also class III ADH and govern elimination rate of blood ethanol. Leg. Med. (Tokyo), 5: 202-211.

Kong, X., M. Guo, Y. Hua, D. Cao and C. Zhang, 2008. Enzymatic preparation of immunomodulating hydrolysates from soy proteins. Bioresour. Technol., 99: 8873-8879.

Lin, F., L. Chen, R. Liang, Z. Zhang and J. Wang, 2010. Pilot-scale production of low molecular weight peptides from corn wet milling byproducts and the antihypertensive effects *in vivo* and *in vitro*. Food Chemistry, 124: 801-807.

Malinowska, B., D. Napiorkowska-Pawlak, R. Pawlak, W. Buczko and M. Gothert, 1999. Ifenprodil influences changes in mouse behaviour related to acute and chronic ethanol administration. Eur. J. Pharmacol., 377: 13-19.

Tupala, E. and J. Tiihonen, 2004. Dopamine and alcoholism: Neurobiological basis of ethanol abuse. Prog. Neuropsychopharmacol. Biol. Psychiatry, 28: 1221-1247.

WHO, 2004. Global status report on alcohol 2004. World Health Organization Geneva.

Xu, B.J., B.J. Xu, Y.N. Zheng, B.J. Xu and Y.N. Zheng *et al*., 2005. Natural medicines for alcoholism treatment: A review. Drug Alcohol Rev., 24: 525-536.

Xu, L., L. Liu, X. Li, Y. Qiao, X. Wu and X. Zhang, 2002. Study on the preparation of corn functional oligopeptides and its SOD-like activity. Chinese J. Biochem. Pharma., 23: 78-80.

Yamaguchi, M., F. Nishikiori, M. Ito and Y. Furukawa, 1997. The effects of corn peptide ingestion on facilitating alcohol metabolism in healthy men. Biosci. Biotechnol. Biochem., 61: 1474-1481.

Yamaguchi, M., M. Takada, O. Nozaki, M. Ito and Y. Furukawa, 1996. Preparation of corn peptide from corn gluten meal and its administration effect on alcohol metabolism in stroke-prone spontaneously hypertensive rats. J. Nutr. Sci. Vitaminol., 42: 219-231.

Yang, Y., G. Tao, P. Liu and J. Liu, 2007. Peptide with angiotensin I-converting enzyme inhibitory activity from hydrolyzed corn gluten meal. J. Agric. Food Chem., 55: 7891-7895.

Zhang, Z., S. Li, J. Jiang, P. Yu, J. Liang and Y. Wang, 2010. Preventive effects of Flos Perariae (Gehua) water extract and its active ingredient puerarin in rodent alcoholism models. Chin. Med., 5: 36-36.