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## Protective Effect of Cynanchum Wilfordii Root Crude Extract on Acetaminophen-Induced Liver Toxicity

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#### Abstract

The objective of this study was to investigate the protective effect of Cynanchum wilfordii root crude extract (CWR) on acetaminophen(AAP) overdose-induced liver damage. We tested the hepatoprotective effect of CWR in a normal liver cell line(Chang cell) using cell viability assay and western blot analysis. The protective effect of CWR was determined using hematoxylin and eosin staining and immunohistochemistry in vivo. Our results showed that the CWR significantly prevented AAP–induced cell death through the extra cellular signal-regulated kinase 1/2(ERK1/2) activation. AAP-induced liver damage in mice was significantly reduced when CWR was administered at a dose of 100 mg/kg/day(for 7 days) in saline solution. CWR regulated the expressions of inflammation-related proteins such as tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) and inducible nitric oxide synthase(iNOS). These data suggest that CWR presents hepatoprotective effect via the activation of ERK1/2 and the down-regulation of TNF- $\alpha$  and iNOS, which are closely associated with anti-inflammatory responses. Moreover, these findings suggest that CWR can act as a potential therapeutic agent in the treatment of liver damage.

[Keywords] Cynanchum Wilfordii, Crude Extract, Liver, Acetaminophen, Anti-Inflammation

#### **1. Introduction**

The liver is an essential organ that plays a vital metabolic role in detoxification and maintenance of blood glucose levels. Although this organ exhibits excellent regenerative capacity, it is highly vulnerable to chemical-induced damage and the accompanying oxidative stress. Liver failure, which is often associated with necrosis, is due to continuous and abnormal oxidative stress and drug-induced liver damage[1]. In western countries, approximately 50% of the total incidence of acute liver failure(ALF) is caused by drug abuse.

Acetaminophen(AAP), also known as paracetamol, has been widely prescribed for the treatment of high fever and pain. Yet, AAP can be harmful and lead to liver and kidney damage in children. Despite the fact that AAP is one of the leading causes of liver-related morbidity and mortality, most patients are unaware of the risks associated with AAP overdose[2][3]. Acetaminophen metabolism involves the formation of toxic intermediate by-products such as N-acetyl-p-benzoquinone imine(NAPQI), a highly reactive metabolite. In an acute overdose, this toxic metabolite induces inflammatory responses and hepatic necrosis[4].

Nitric oxide(NO) exhibits both harmful and beneficial functions in cellular metabolism. NO generation plays a crucial role of maintaining immune-homeostasis in cellular metabolism against bacterial infections. How-

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ever, they can also induce liver-cell dysfunctions because excessive NO generation induces cell death, cellular damage, and progression of diseases through the activation of immune responses[5].

Thus far, approximately 300 species of Cynanchum wilfordii belonging to the Cynanchum genus have been established. C. wilfordii species have been used in Korean traditional medicine to improve stamina[6]. Moreover, several studies have proven their effectiveness in preventing endothelial dysfunction, as well as their anti-fungal and antioxidant properties. However, the effect of C. wilfordii in maintaining the protection and regeneration in liver dysfunction has not yet been established. In this study, we investigated the effects of C. wilfordii in the regulation of cellular dysfunction as we expect that its combined treatment can be efficient in counteracting liver failure.

#### 2. Methods

#### 2.1. Chemicals

Cell culture reagents were purchased from Gibco BRL(Gaithersburg, MD). The EZ-cytox Cell Viability Assay and Vectastain ABC Kits were obtained from Daeil Lab Service(Seoul, Korea) and Vector Laboratories(Burlingame, CA, USA), respectively. The following chemicals and antibodies used in this study - COX-2, iNOS, TNF- $\alpha$ , APE1/Ref-1, and GAPDH - were purchased from Santa Cruz(Santa Cruz, MA). AAP and all other reagents were purchased from Sigma(St. Luis, MO).

#### 2.2. Design of experiments

We performed the EZ-cytox Cell Viability Assay to evaluate cell viability, western blot to test the expression levels of proteins, and histopathological assay to determine the hepatoprotective effect of CWR.

#### 2.3. Procedure

#### 2.3.1. Plant materials and water extraction

C. wilfordii was obtained from Dongguk University Oriental Hospital, Korea. One-hundred grams of the plant root were blended, and the crude powder was precipitated with 1000 mL of sterile deionized water at 100°C for 3 h. The aqueous extracts were concentrated and evaporated at 60°C under vacuum conditions. The extract was dissolved in 50 mL of sterile deionized water. The aqueous extract was lyophilized by freeze-drying at -60°C. Finally, we obtained 27.4% powder (27.4 g) from the plant root.

#### 2.3.2. Cell culture and cell viability assay

Chang cells were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin and maintained in a humidified atmosphere containing 5% CO2 at 37°C. A total of 5  $\times$  104 cells were seeded in a 96-well microplate, and the cells were incubated with different concentrations of the C. wilfordii extract(CWR; dissolved and diluted in DMEM) for 24 h. Thereafter, cell viability was measured by carrying out ELSIA using the EZ-cytox Cell Viability Assay Kit, and the data were acquired using an ELISA reader according to the manufacturer's instructions.

#### 2.3.3. Western blot

The cell lysates were obtained from the Chang cells after a defined culture period. Twenty micrograms of proteins were separated using SDS-PAGE on gels containing 12% acrylamide. Then, the proteins were transferred onto a polyvinylidene difluoride(PVDF) membrane(Amersham Pharmacia Biotech) at 4°C. This membrane was then soaked overnight in 5% skim milk at 4°C. Subsequently, the membrane was washed using Tris-buffered saline containing 0.1% Tween 20. The membrane was incubated with t-ERK1/2, p-ERK1/2 and GAPDH antibodies(1:1000 dilution). After incubation with the corresponding secondary antibodies, the membrane was analyzed using chemiluminescent reaction (ECL plus kit, Amersham Pharmacia Biotech), and proteins were visualized and analyzed using Image J Software.

#### 2.3.4. Animal care

Six-week-old male ICR mice were obtained from Orient Bio. Inc., Korea. Prior to the experiments, all animals were allowed to acclimatize to the new environment for one week (at room temperature, 24 ± 2 °C; humidity, 50 ± 15%; and 12-h light/dark cycle). All experiments and animal care were conducted in conformity with the institutional guidelines of the Dongguk University. To test the hepatocytoprotective effect of CWR, the animals were randomly divided into three different groups(n = 8 mice/group): control group (normal mice), CWR- and acetaminophentreated group, and acetaminophen treated group. C. wilfordii root crude extract(CWR; 100 mg/kg/day in normal saline) was administered orally every day for 7 days, following a regular schedule. Liver injuries were induced by acetaminophen(diluted in normal saline). Acetaminophen was administered by a single intraperitoneal injection at a dose of 400 mg/kg, 1 h after the last CWR treatment.

#### 2.3.5. Histological assay

The mice were anesthetized with sodium pentobarbital solution and killed. Their livers were isolated and fixed in 10% formalin for 24 h. The livers were embedded in paraffin, and 6-m-thick tissue sections were stained with hematoxylin-eosin(H&E) stain. Some sections were used for Wright's stain to observe blood cells in the liver. Some of the prepared sections were used for immunostaining assays. Samples were treated with 3% H2O2 for 5 min to inactivate endogenous peroxidase, then blocked with 10% normal serum for 1 h at room temperature, and incubated overnight at 4°C with primary antibodies against tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) and inducible nitric oxide synthase(iNOS). The following day, the sections were washed and incubated with the corresponding secondary antibodies for 1 h at room temperature. The Vectastain ABC Kit was used to implement the avidin-biotin complex interaction, in accordance with the manufacturer's instructions. Signal development was carried out in a substrate solution of 0.05% DAB, and the slides were counterstained with hematoxylin. The sample sections were examined using a light microscope (Olympus BX50, Japan) at 200 X magnification.

#### 2.3.6. Statistical analysis

Data are expressed as the mean ± standard error of the mean(S.E.M.) in the indicated number of experiments. Statistical analysis of

the data was performed using student's t test for comparisons between pairs of groups using GraphPad prism(GraphPad Software, San Diego, CA, USA). P values < 0.05 were considered significant.

### 3. Results

#### 3.1. Effect of CWR on cell viability and ERK 1/2 phosphorylation in acetaminophen-stimulated Chang cells

We assessed whether CWR can regulate ERK1/2 phosphorylation in AAP-induced Chang cells. In this study, CWR showed no cytotoxicity at doses up to 200 µg/mL. AAPstimulated Chang cells were treated with varying concentrations of CWR(100 and 200 µg/mL) for 24 h. As shown in <Figure 1A>, AAP significantly reduced cell viability, whereas CWR inhibited AAP-induced cell death in Chang cells. Next, the cell lysates were analyzed using western blot. Cell viability was significantly induced by CWR. The expression level of ERK1/2 phosphorylation was evaluated by normalizing the intensity of the bands against the control gene, ERK expression. As shown in <Figure 1B>, AAP-stimulated Chang cell treated with CWR at concentrations of 100 and 200 µg/mL significantly increased ERK1/2 phosphorylation to 150% and 190%, respectively.

Figure 1. Effect of CWR on AAP-stimulated Chang cell (A) The cells were treated with the presence or absence AAP and CWR concentrations(100, and 200 μg/mL). Cell viability was determined via cell viability assay. (B)Cells lysates were examined via immunoblot analysis with specific antibodies such as anti-ERK1/2 phosphorylation(p-ERK), anti-total ERK1/2(t-ERK) and anti-GAPDH (GAPDH). The graphs represent the intensity of the bands relative to the AAP-treated group. Results are presented as means ± standard errors, and are representative of three independent experiments. \*P < 0.05 versus untreated group.</p>



#### 3.2. Protective effect of CWR on acetaminophen-induced liver damage in mice

To examine the protective effect of CWR against acetaminophen-induced liver injury, we performed the histological analysis using H&E staining in a mouse model. In the group pretreated with CWR through oral administration(for 7 days) before overdosing with AAP, we observed decreased AAP-induced centrilobular degeneration and necrosis. As shown in <Figure 2>, tissue samples treated with acetaminophen(400 mg/kg) exhibited severe necrosis with vacuole formation in the hepatocytes. In contrast, samples pretreated with CWR(100 mg/kg) showed the morphology similar to normal naïve hepatocytes when compared to the untreated group.

Figure 2. Effect of CWR in acetaminophen-induced liver injury in mice Liver sections were stained with H&E. The untreated group of animals was given water only(Untreated group), one group of animals was treated with acetaminophen(AAP), the other group was treated with acetaminophen after pretreatment with 100 mg/kg of CWR for 7 day(AAP+CWR). The photomicrographs show a magnification of ×200(upper panel) and ×400 (lower panel); bar = 100 μm.



#### 3.3. Effect of CWR on iNOS and TNF-α expression in AAP-induced injury of liver tissue

To examine possible correlations between hepatocyte protection and regulation of redox, we studied the expression levels of inflammation-related signaling molecules, iNOS and TNF- $\alpha$ , in AAP-induced liver failure in mice. We observed the expression levels of iNOS and TNF- $\alpha$  to be 100% in the AAPtreated group. CWR pretreatment decreased their expression by 70%, and 80% respectively.

**Figure 3.** Expression of iNOS and TNF- $\alpha$  in acetaminophen-induced liver failure and CWR-treated liver(A). The graph shows the expression levels of iNOS and TNF- $\alpha$  obtained from A. Expression changes are relative to the acetaminophen treated group(B). The results are representative of more than three independent experiments. Results are presented as the means  $\pm$  standard errors, \* *P* < 0.05 versus acetaminophen-treated group.



#### 4. Discussion and Conclusion

Reactive oxygen species(ROS) are bifunctional catalyst molecules exhibiting both beneficial and harmful effects on the cells. ROS can support vital cellular activities such as induction of transcription factors and accommodation of the receptor signals. However, an excessively increased levels of ROS in the cells lead to oxidative stress that can induce extensive cell damage such as DNA destruction, lipid peroxidation, and protein degeneration[7]. Overdose of AAP is associated with the ROS generation signals[1]. Dahlin et al. supported the idea that following acetaminophen overdose, AAP metabolism leads to the formation of a toxic intermediate such as Nacetyl-p-benzoquinone imine (NAPQI), which causes acute liver failure[3]. Furthermore, over dose of AAP induces cell death and ERK1/2 phosphorylation in Chang cells[8]. Especially, a defense mechanism is associated with ERK1/2 activation. We also confirmed that CWR regulates ERK 1/2 phosphorylation. Therefore, we suggest that CWR may have an antioxidant effect.

AAP overdose-induced liver damage is highly relevant to the inflammatory signaling pathway that includes components such as TNF-α signal and pro-inflammatory cytokines[2][5]. In particular, up-regulation of TNF- $\alpha$  and expression of iNOS have been observed in histological sections of liver lesions. The signal cascades and transductions are triggered by phosphorylation of proteins. Especially, the extracellular signal-regulated kinases 1/2(ERK1/2), which are activated by cell survival signal, participate in liver damage and regeneration [6]. We also confirmed that CWR regulates TNF- $\alpha$  and iNOS. The results suggest that CWR is one of the candidates for combined prescription against AAP-induced liver damage and can significantly regenerate liver injuries.

In conclusion, we clearly demonstrate the hepatocyte-protective effect of CWR in vitro and vivo. Our results suggest that CWR down-regulates ERK1/2 phosphorylation that is attributable not only to the suppression of iNOS but also to the regulation of TNF- $\alpha$  expression. Therefore, CWR is a promising therapeutic agent against acute and chronic liver failure induced by AAP over-dosage.

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## Effects of Band Resistance Exercise and Balance Training on Improvement of SPORTS Injury and Performance of Men's University Bowling Players

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#### Abstract

This study was to investigate the effects of band resistance exercise and balance training on the improvement of sports injury and performance in the men's university bowling players. To achieve this purpose, the athletes who have been registered as a member of the Korea Bowling Association with more than 5 years of athletic career were selected, and after observing the enhancement of sports injury and performance through the band resistance exercise and balance training, the following conclusion is obtained. First, As for the Average score, there were significantly increased in score of the athletes in 2014 before the training was 191.26, while the average score of the athletes in 2015 after the training was 196.88, which increased by 5.62 compared to the pretest. Second, as for the Frequency of Sports Injuries by Period, a total of 196 injuries occurred before training in 2014, of which 86.8% occurred during the preparation season. A total of 94 injuries occurred in 2015 after the training, which was significantly reduced compared to pre-training, yet 76.6% of the injuries occurred during the preparation season. The athletes' injury a rate was reletively higher during the preparation season compared to competition season and break season. Third As for the Sports Injury Type, there were decreased increased in 2015. As for the Sports Injury Type and Sports Injury Area, there were Overall improvement increased in 2015. However, there is a need for more specific exercise programs to be developed for the continual improvement of athletic performance in band resistance exercise and balance training along with further studies to confirm the physiological benefits of those programs.

#### [Keywords] Band Resistance Exercise, Balance Training, Sports Injury, Bowling, Men's University Bowling

Players

#### 1. Introduction

It is required for athlete to participate in intense, continuous and repeated training to improve physical strength and specific skills, and athletes who have to exert a intense training may experience a physical imbalance and a decrease in performance due to an incorrect exercise habits and long-term one side movement[1][2]. Among them, trunk rotational exercise of bowling athletes affects body malalignment syndrome in posture and walking, and continuous unilateral movement of bowling athletes causes various sports injury due to a physical imbalance. In addition, physical imbalance hinders athlete's records, increases the risk of injuries to athletes and thereby slows the recovery rate of injuries that have occurred[3]. The improvement of complex factors required in bowling is related to improvement of performance directly and generally decisive factors that determine the performances are physical strength, specific skills and willpower, yet among them physical strength factor is the most basic, which is based on muscular strength required for bowling, lower body strength is especially needed since bowling is a continuous repetition of pitching while sliding a short distance in a short time[5]. Thus strengthening of the leg muscles is regarded as the first factor affecting the performance. In addition, among various functions, leg strength plays an important role especially in effectively controlling the center of the body[6].

A weight has been used often for the resistance exercise to improve the muscle strength, yet since weight training has a high risk of injury, so band resistance training is used nowadays. Band resistance exercise is especially useful in that it is easy to carry, easy to perform for everyone, has constant tension, and there is less injury unlike weight training[7][8]. Moreover, the characteristic of the band resistance movement is that it can provide a continuous resistance while any movement is performed in every human movement directions, so that it is especially effective in the multi-directional movement of daily life and muscle strength[9]. The spiral and diagonal movements are very similar to the movements caused by physical functional activity[10], and so if these movements can be combined with the band resistance exercise, a functional resistance exercise with a centrifugal force, centripetal force and isotonic contraction can be made. For bowling like this, balance and muscle strength is the most important, and balance movement is the ability to maintain and adjust to the center of gravity of the support surface[11], which is a process of maintaining posture stability continuously, and also influences on the performance of stability, weight control and walking[12], and balance movement exercise can enable optimal nerve adaptation which includes coordination of intramuscular and between the muscles. Intramuscular coordination is the ability of the muscles to participate in the movement unit and to have an enhanced movement unit identity[13].

As such, bowling is a typical unilateral exercise that causes body imbalance, which has a very high risk of sports injury. In addition, bowling skills are very sophisticated, so if posture stability is not achieved, then professional physical movement and technique can't be performed. Therefore, a bowling requires a training method to stabilize professional physical strength and technical movements.

Therefore, enhancement of balance and its function through band resistance exercise and balance training is a very important physical approach to bowling athletes. That is, if the band resistance exercise and the balance training are continuously applied, it is expected that these exercises will help the bowler to stabilize the body and to prevent injuries caused by physical imbalance, which will help improve the performance ultimately. Thus, the purpose of this study is to identify the effect of 12 week band resistance exercise and balance training on male college bowling athletes.

#### 2. Materils & Methods

#### 2.1. Subject of study

This study is conducted from March 2016 to June 2016 for J university bowling athletes, and the athletes who have been registered as a member of the Korea Bowling Association with more than 5 years of athletic career are selected. The 12 subjects who are willing to participate and fully understand the purpose of this research are selected. The physical characteristics of the subjects are shown in <Table 1>.

 Table 1. Physical characteristics of subjects mean±SD.

N	Age (Years)	Height (Cm)	Weight (Kg)	BMI(kg/m²)	Percent body Fat(%)
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#### 2.2. Exercise program

#### 2.2.1. Band resistance exercise

A band resistance exercise was performed 3 times a week for 12 weeks, and total 60

 Table 2. Band resistance exercise program.

minutes of exercise consist of 10 minutes of preparation, 40 minutes of exercise and 10 minutes of warm down exercise. The band resistance exercise program is shown in <Table 2>.

ltem	Week	Intensity (Band color)	Program	Time	Frequency	Rest
1	1~4	Medium (Red)	Plantar flexion Dorsi flexion			
2	5~8	Heavy (Green)	Inversion Eversion Plantar flexion	30min	15times ×	30sec
3	9~12	Extra heavy (Blue)	Dorsi flexion Inversion Eversion		4sets	

#### 2.2.2. Balance training

The subjects of this study was participating in the college exercise program, and this study's program was performed during the off season in the winter. In other words, balance training was not carried out independently in specific time but additionally after the normal training.

The balance training was carried out 3 times a week for 12 weeks, and total 60

minutes of exercise consist of 10 minutes of preparation, 40 minutes of exercise and 10 minutes of warm down exercise. The balance training consisted of three steps: the first 4 weeks of exercise program of securing the basic balance and strength, the next four weeks of securing dynamic balance and enhancing muscle strength, and the last four weeks of combining dynamic balance with the muscle strength. The balance training program of this study is shown in <Table 3>.

				Rest		
Stage	Ex	ercise program	1~4 Week	5~8 Week	9~12 Week	Motion frequency
			(reps/set)	(reps/set)	(reps/set)	
Warm-up (10 min)		Skipping Side steps Carioca Back slaps	10/3	10/3	10/3	RPE 8~10
Work-out	Prone	1. elbow stand	10/2	10/3	10/5	RPE

Table 3. Balance exercise program.

(40min)	position	2. elbow stand				11~13
	Supine position	left(light) leg up 3. hand stand left(light)				
	Lateral position	leg up				
	Elastic band train- ing	1. Squirt 2. Lunge 3. Side lunge				
	Balance ball train- ing	<ol> <li>One feet balancing</li> <li>Two feet balancing</li> <li>Walking in balance ball</li> <li>Run in balance ball</li> </ol>				
Cool-down (10min)	Ham string stretch Shoulder stretch Calf stretch Adductor stretch Quadriceps stretch It band stretch		10/3	10/3	10/3	RPE 8~10

#### 3. Measure and Method

#### **3.1.** Performance evaluation

To asess the performance of bowling athletes at J University in C region, an average score per game was calculated based on 8 major championships in 2014 and 8 major championships in 2015.

#### 3.2. Sports injury investigation

The subjects, who had a medical treatment due to orthopedic injury during a season or who were absent from a team training, were selected for this study. In cases of medical examination, the cases were limited to new patients while excluding existing patients or medical treatment for simple examination, and in cases of absence from team training, cases of injury recurrence after recovery were included. Records were collected with accurate date and time of injury, area of injury, diagnosis and position of injured player from each club's medical trainer. All data were based on medical records and medical logs.

The collected data were classified as follows to analyze.

1)Season: preparation season / championship season / break season 2)Injured area: ankle, knee, quadriceps, hamstring, foot, shoulder, neck, elbow, wrist, finger, waist, hip joint, trunk, tibia, calf, achilles tendon, ect.

3)Type of injury: sprain, strain, muscle tear, ligament tear, myalgia, fracture, meniscus injury, chondral injury, HIVD, SLAP, tendinitis, impingement syndrome, ect.

#### 4. Data Process

For the data analysis of this study, the mean and standard deviation of all collected data were calculated using SPSS 20.0(window statistical package), and frequency analysis was performed for injury rate. Paired t-test was carried out for significance test before and after experiment within the group. At this time, the significance level was p<.05.

#### 5. Results

#### 5.1. Average score

Changes in the average score as a result of the band resistance exercise and balance training are shown in <Table 4>. For the changes in the average score due to the band resistance exercise and balance training, the average score of the athletes in 2014 before the training was 191.26, while the average score of the athletes in 2015 after the training

was 196.88, which increased by 5.62 compared to the pre-test.

Table 4. The changes of average score.

	Average score	t	р
2014	191.26 ± 12.73	1.699	125
2015	196.88 ± 4.47	-1.088	.135

#### 5.2. Sports injury

#### 5.2.1. Frequency of injury by season

As shown in <Table 5>, for the difference in frequency of injuries by season in relation to the band resistance exercise and balance training, a total of 196 injuries occurred before training in 2014, of which 86.8% occurred during the preparation season. A total of 94 injuries occurred in 2015 after the training which was significantly reduced compared to before training, yet 76.6% of the injuries occurred during the preparation season. The athletes' injury rate was relatively higher during the preparation season compared to competition season and break season.

**Table 5.** Frequency of sports injuries by period.

	2014 Year		2015 Year		
	Frequency	%	Frequency	%	
Preparation	171	86.8	72	76.6	
Match period	20	10.2	20	21.3	
Rest period	5	2.5	2	2.1	
Total	196	100	94	100	

#### 5.2.2. Type of injury

The differences of type of injury in relation to the band resistance exercise and balance training are shown in <Table 6>. As shown in <Table 6>, for the difference of the type of injury in relation to the band resistance exercise and balance training, there were 148 cases of sprains which is 75.5% of the all injuries occurred in 2014 before the training, followed by 15.3% of contusion, 7.7% of cartilage torn and 1.5% of fracture, and in 2015 after the training, there were 71 cases of sprains which is 75.5% of the injuries, followed by 22.3% of contusion and 2.1% of cartilage torn. However, there was no fracture before and after the training.

	2014 Year		2015 Year	
	Frequency	%	Frequency	%
Fracture	3	1.5	0	0
Sprains	148	75.5	71	75.5
Contusion	30	15.3	21	22.3
Cartilage	15	7.7	2	2.1
Total	196	100	94	100

Table 6. Sports injury type.

#### 5.2.3. Injured area

The difference of the injured area in relation to the band resistance exercise and balance training is shown in <Table 7>. As shown in <Table 7>, for the difference of the injured area in relation to the band resistance exercise and balance training, there were 41 cases of wrist injures which is 20.9% of the all injuries occurred in 2014, followed by 16.8% of waist, 12.8% of shoulder, 12.2% of elbow and 12.2% of hip, and for the injuries in 2015 after the training, 21.3% were wrist, followed by 19.1% of waist and 16% of elbow. The order of the injured parts before and after the training was the same, but the number of injuries decreased greatly overall.

	2014 Year		2015 Year		
	Frequency	%	Frequency	%	
Neck	0	0	0	0	
Chest	8	4.1	0	0	
Waist	33	16.8	18	19.1	
Hip	24	12.2	8	8.5	
Knee	16	8.2	8	8.5	
Ankle	17	8.7	12	12.8	
Shoulder	25	12.8	11	11.7	
Elbow	24	12.2	15	16.0	
Wrist	41	20.9	20	21.3	
Groin	8	4.1	2	2.1	
Total	196	100	94	100	

#### **Table 7.** Sports injury area.

#### 6. Discussion

Nowadays sports can be called as a sports of a science. It is because athletes try to improve their performance by using scientific methods even from the training. However, despite the enhancement of equipment safety, coaching ability and knowledge of physical condition as a result of development of science and technology, sports injuries are increasing in all sports[14]. Bowling has a very high rate of sports injury as well. Bowling is deemed as a typical unilateral exercise that causes physical imbalance, which can lead to muscle dysfunction and pain, and may cause disruption of life quality such as neurological atrophy, kidney weakness and fatigue[15]. In addition, a decline in competitiveness due to an injury leads to an athlete's and team's performance as well. In order to make up for this, posture stabilization program and balance training program of bowling athletes are applied to improve performance and prevent injuries. Thus, the purpose of this study is to verify the effects of the band resistance exercise and balance training of men's college bowling athletes for 12 weeks on sports injury and performance.

A bowling is a sport in which a good balance and rhythmic movement is especially important, and an excellent performance is achieved when technical skills such as swing, step, release, turning and lifting are harmonized with the ability to adjust the direction of the ball. In order to perform this sophisticated and exact movement, a repeated training as well as appropriate morphological properties are needed[16][17][18]. In order to achieve correct posture and physical balance, a training method that can correct physical imbalance should be applied, and the balance training is used as one of the methods. The balance training maximizes the effect of relaxation by relaxing the time that the nerve signal is delivered to the brain through the homaxial contraction of the muscle for each movements for more than 6 seconds. Through this effect, the balance training helps to relieve tension, strengthen muscle, improve flexibility and posture, and reduce the risk of injury[19]. For the difference in frequency of injuries by season in relation to the band resistance exercise and balance training, a total of 196 injuries occurred before training in 2014, of which 86.8% occurred during the preparation season. A total of 94 injuries occurred in 2015 after the training which was significantly reduced compared to before training, yet 76.6% of the injuries occurred during the preparation season. The athletes' injury rate was relatively higher during the preparation season compared to competition season and break season. Among them the frequency of injuries was especially higher during the preparation season than the championship season, but it is because most athletes endure the pain and injury during the competition season to participate in the match.

For the difference of the type of injury in relation to the band resistance exercise and balance training, there were 148 cases of sprains which is 75.5% of the all injuries occurred in 2014 before the training, followed by 15.3% of contusion, 7.7% of cartilage torn and 1.5% of fracture, and in 2015 after the training, there were 71 cases of sprains which is 75.5% of the injuries, followed by 22.3% of contusion and 2.1% of cartilage torn. However, there was no bone fracture before and after the training. For the difference of the injured area in relation to the band resistance exercise and balance training, there were 41 cases of wrist injures which is 20.9% of the all injuries occurred in 2014, followed by 16.8% of waist, 12.8% of shoulder, 12.2% of elbow and 12.2% of hip, and for the injuries in 2015 after the training, 21.3% were wrist, followed by 19.1% of waist and 16% of elbow. The order of the injured areas before and after the training was the same, but the number of injuries decreased greatly overall. This is consistent with the studies that the balance training program is implemented extensively to protect athletes from various injuries that may occur during exercise, and the balance

ability contributes to reduce the frequency rate of the injuries to athletes [20][21]. In addition, even if the imbalanced posture, repeated movements and muscle overload may cause deformation of the muscle skeletal system, the same movements has to be carried out repeatedly for the better performance[22], which results in physical changes and defects that have a continuous effect on performance[23]. A muscle strengthening training using a band has positive effects on stabilizing posture[12], reducing physical pain[24], increasing the stability of the ankle on an unstable surface, and activating abdominal muscles, back muscles, leg muscles and flexor muscles of knees. In addition, statistically significant difference was found in the repeated throwing training since leg muscle strength and endurance is especially required to maintain sliding movement during the throwing stage[25]. In this study, the average score of the athletes in 2014 before the training was 191.26, while the average score in 2015 after the training was 196.88, which increased by 5.62 compared to pre-training.

Putting all the above results together, the band resistance exercise and balance training help improve sports injury and performance of bowling athletes, and an excellent performance is achieved by harmonizing posture stabilization, technical skills through balanced posture and the ball adjusting ability. Furthermore, in order to perform sophisticated and stabilized movement like above, a repeated training as well as a development of training suitable for bowling athletes are required.

Therefore, this study, which was conducted to investigate the effects of 12 weeks band resistance exercise and balance training on male college bowling athletes, verified that the program helped to improve sports injury and performance.

#### 7. Conclusion

The purpose of this study was to find out the effects of the band resistance exercise and balance training on sports injury and performance of male college bowling athletes, and verifies that an improvement of sports injury and performance can be achieved through the band resistance exercise and balance training, and thus provides basic data for development of training method to prevent injury and enhance performance by stabilizing balance of bowling athletes.

To achieve this purpose, the athletes who have been registered as a member of the Korea Bowling Association with more than 5 years of athletic career were selected, and after observing the enhancement of sports injury and performance through the band resistance exercise and balance training, the following conclusion is obtained.

1. For the changes of an average score after the band resistance exercise and balance training, the average score of the athletes in 2014 before the training was 191.26, while the average score of the athletes in 2015 after the training was 196.88, which increased by 5.62 compared to the pre-test.

2. For the difference in frequency of injuries by seasons after the band resistance exercise and balance training, a total of 196 injuries occurred before training in 2014, of which 86.8% occurred during the preparation season. A total of 94 injuries occurred in 2015 after the training, which was significantly reduced compared to pre-training, yet 76.6% of the injuries occurred during the preparation season. The athletes' injury arate was reletively higher during the preparation season and break season.

3. For the difference of the type of injury after the band resistance exercise and balance training, there were 148 cases of sprains which is 75.5% of the all injuries occurred in 2014 before the training, followed by 15.3% of contusion, 7.7% of cartilage torn and 1.5% of fracture, and in 2015 after the training, there were 71 cases of sprains which is 75.5% of the injuries, followed by 22.3% of contusion and 2.1% of cartilage torn. However, there was no fracture before and after the training.

4. For the difference of the injured area after the band resistance exercise and balance training, there were 41 cases of wrist injures in 2014 which is 20.9% of the all injuries, followed by 16.8% of waist, 12.8% of shoulder, 12.2% of elbow and 12.2% of hip, and for the injuries in 2015 after the training, 21.3% were wrist injuries, followed by 19.1% of waist and 16% of elbow. The order of the injured parts before and after the training was the same, but the number of injuries decreased greatly overall.

In conclusion of this study, the band resistance exercise and balance training are verified to be effective for improving the sports injury and performance of college male bowling athletes. In addition, in order to perform sophisticated and stabilized movement through improving physical imbalance and posture, a repetitive training as well as a development of training suitable for bowling athletes are needed.

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## Anti-Inflammatory Effect of Cynanchone a in Lipopolysaccharide-Induced RAW 264.7 Cells

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#### Abstract

It has been reported that Cynanchum wilfordii exhibits anti-oxidant, and anti-cancer activities. However, little is known about biological activity of cynanchone A, an acetophenone isolated from C. wilfordii. In the present study, we investigated the anti-inflammatory effect and the underlying molecular mechanisms of cynanchone A in lipopolysaccharide(LPS)-induced RAW 264.7 cells. During the LPS-induced inflammatory process in RAW 264.7 macrophages, nitric oxide(NO) and prostaglandin E2(PGE2) productions increased. Cynanchone A significantly decreased the protein levels of NO and PGE2 in LPS-treated RAW 264.7 macrophages. Moreover, cynanchone A significantly reduced the elevated the expression levels of inducible nitric oxide(iNOS), cyclooxygenase-2(COX-2) and tumor necrosis factor-a(TNF-a) in LPS-stimulated RAW264.7 cells. Consequently, cynanchone A exerted an anti-inflammatory action through inhibiting nuclear factor-kappa B(NF-κB) activation. Taken together, our results show that the anti-inflammatory properties of cyanchone A are involved in the down-regulating pro-inflammatory cytokines and regulating NF-kB signal pathway in LPS-stimulated RAW264.7 cells.

[Keywords] Cynanchum Wilfordii, RAW264.7, Anti-Inflammatory, Anti-Oxidant, Cyanchone A

#### **1. Introduction**

Inflammation is a process that involves multiple factors that act in concert. The ingress of leukocytes into sites of inflammation is an important aspect of the pathogenesis of inflammatory conditions[1]. For example, macrophages are recruited to inflammatory sites, and are activated by various signals that stimulate many intracellular cascades of cytokines and chemokines[2]. In macrophages, lipopolysaccharide(LPS), a well-known endotoxin, increases the productions of inflammatory cytokines, such as, tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) and interleukin-6, and inflammatory mediators, such as, nitric oxide(NO) and prostaglandin E2(PGE2), which are synthesized by inducible NO synthase(iNOS) and cyclooxygenase-2(COX-2), respectively[3][4].

NF-KB is a transcriptional factor that plays a pivotal role in immune and inflammatory responses via the regulation of genes that encode pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes like COX-2 and iNOS[5][6]. Under normal conditions, NF-κB is sequestered in the cytoplasm as an inactive complex, due to binding with IkB proteins [7]. In the classic pathway, activation of NF-KB, especially the most abundant form, p50/p65 heterodimer, depends on the phosphorylation of its endogenous inhibitor IKB, mainly by IKB kinases(IKKs)[8][9]. This leads to ubiquitination and proteasomal degradation of IkB. The liberated NF-kB dimer then translocates to the nucleus, where it activates specific target genes[10][11]. Several studies have reported that the activation of NF-κB is triggered by mitogen-activated protein kinases(MAPKs), including extracellular signal-regulated protein

kinases 1/2, p38 MAPK kinase, and JNK[12]. Other reports have shown that the negative regulation between NF- $\kappa$ B and MAPK[13]. The relationship between NF- $\kappa$ B and MAPKs are complex and depends on the cell type and stimulus.

The roots of Cynanchum wilfordii have traditionally been used to help liver and kidney, to strengthen the bone and muscle, and to invigorate in Korea. In previous studies have shown that the extract and fractions of this herb have various pharmacological activities, including the effect of scavenging against free radicals, enhancing immunity, reducing high serum cholesterol, and having anti-tumor activity[14]. Cynanchone A is acetophenone isolated from roots of C. wilfordii. Since the first discovery of Cyanachone A in 1999, these biological effects were still poorly understood. Therefore, as a part of our on-going screening program to evaluate the anti-inflammatory potentials of natural compounds, we selected Cynanchone A, and investigated its anti-inflammatory effects and the mechanism involved in RAW264.7 macrophage cells, which can be stimulated by LPS to mimic conditions of infection and inflammation.

#### 2. Materials and Methods

#### 2.1. Cell culture and sample treatment

The RAW 264.7 murine macrophage cell line was obtained from the Korea Cell Line Bank(Seoul, Korea). These cells were grown at 37°C in DMEM medium supplemented with 10 % FBS, penicillin(100 units/ml), and streptomycin sulfate(100  $\mu$ g/ml) in a humidified atmosphere of 5% CO<sub>2</sub>. Cells were incubated with Cynanchone A at concentrations of 25, 50, and 100  $\mu$ M, and then stimulated with LPS(1  $\mu$ g/ml) for the indicated time.

# 2.2. Measurement of cell viability by MTT assay

RAW 264.7(1x10<sup>4</sup>/well) cells viability studies were performed in 96-well plates. Cynanchone A was dissolved in DMSO, and the DMSO was added to all plates to compensate the same volume of DMSO. After overnight incubation, the test material was added, and the plates were incubated for 24 h. Cells were washed once before adding 50  $\mu$ l of FBS-free medium containing 5 mg/ml of MTT. After 4 h of incubation at 37°C, the medium was discarded and the formazan blue that formed in the cells was dissolved in DMSO 100  $\mu$ l. The optical density was measured at 540 nm.

#### 2.3. Measurement of nitrite in culture media

RAW 264.7 cells were plated at  $2.5 \times 10^5$ cells/ml in 24 well-plates and then incubated with or without LPS(1  $\mu$ g/ml) in the absence or presence of various concentrations(25, 50, and 100  $\mu$ M) of Cynanchone A for 24 h. The nitrite accumulated in culture medium was measured as an indicated of NO production based on the Griess reaction. Briefly, 100  $\mu$ l. of cell culture medium was mixed with 100  $\mu$ l of Griess reagent[equal volumes of 1% (w/v)sulfanilamide in 5%(v/v) phosphoric acid and 0.1%(w/v) naphtylethylenediamine-HCl], incubated at room temperature for 10 min, and then the absorbance at 540 nm was measured in a microplate reader(Perkin Elmer Cetus, Foster City, CA, USA). Fresh culture medium was used as the blank in all experiments. The amount of nitrite in the samples was measured with the serial dilution standard curve of sodium nitrite.

#### 2.4. Determination of PGE2, TNF-α, IL-6, and IL-1 productions

RAW 264.7 cells were pretreated with Cynanchone A(25, 50, and 100  $\mu$ M) for 1 h and then stimulated with LPS(1  $\mu$ g/ml) for 24 h. Levels of PGE2, TNF- $\alpha$ , interleukin(IL)-6, and interleukin(IL)-1 in the culture media were quantified using ELISA kits(R&D Systems, Minneapolis, MN. USA).

# 2.5. Protein extraction and western blot analysis

RAW 264.7 cells were collected by centrifugation and washed once with phosphatebuffered saline(PBS). The washed cell pellets were resuspended in extraction lysis buffer(50 mM HEPES pH 7.0, 250 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 mM Na fluoride, and 0.5 mM Na orthovanadate) containing 5 µg/ml each of leupeptin and aprotinin and incubated with 20 min at 4°C. Cell debris was removed by microcentrifugation, followed by quick freezing of the supernatants. The protein concentration was determined using the Bio-Rad protein assay reagent according to the manufacture's instruction. Forty micrograms of cellular protein was electroblotted onto a polyvinylidene fluoride(PVDF) membrane following separation on a 10% SDS-polyacrylamide gel electrophoresis. The membrane was incubated overnight with blocking solution(5% skim milk) at 4  $\degree$ C, Followed by incubation for 4 h with a primary antibody. The membrane was washed four times with Tween 20/Tris-buffered saline(TTBS) and incubated with a 1:1000 dilution secondary antibody for 1 h at room temperature. The membrane was were again washed three times with TTBS, and then developed by enhanced chemiluminescence(Amersham Life Science.)

#### 2.6. Statistical analysis

Results are expressed as the mean  $\pm$  S.D. of triplicate experiments. Statistical significant values were compared using ANOVA and Dunnett's post-hoc test, and *p*-values of less than 0.05 were considered as significant.

#### 3. Results

#### 3.1. Effects of cynanchone A on on LPS-induced NO and PGE2 production in RAW 264.7 macrophages

The cytotoxic effects of cynanchone A were also evaluated in the presence of LPS using MTT assays, and this compoun d had cytotoxicity at the concentrations of 100 µM <Figure 1>. We investigated t he inhibitory effects of cynanchone A on the LPS-induced productions of the infla mmatory mediators NO and PGE<sub>2</sub> in RA W 264.7 cells. Neither LPS nor samples were added to the control(CON) group. As shown in <Figure 2>, LPS(1  $\mu$ g/ml) in creased NO production by approximately 12-fold, whereas pretreatment with cynan chone A(25, 50, or 100  $\mu$ M) markedly re duced LPS-induced NO production in a d ose-dependent manner. As shown in <Fig

# ure 2>, cynanchone A also dose-depende ntly inhibitedPGE<sub>2</sub> production by LPS.





#### 3.2. Inhibitory effects of cynanchone A on LPS-induced proteins and on the mRNA expressions of iNOS and COX-2

In present study, cynanchone A whether can reduce the LPS-induced NO and PGE<sub>2</sub>, we examined their expression levels by Western blotting and RT-PCR. In unstimulated RAW 264.7 cells, iNOS and COX-2 protein levels were undetectable. However, in response to LPS, their expressions were markedly upregulated. Furthermore, cynanchone A significantly inhibited LPS-induced iNOS and COX-2 in a dose-dependent manner <Figure 3>.

Figure 2. Inhibitory effects of cynanchone A on LPSinduced NO and PGE2 production in RAW 264.7 macrophages. Each value indicates the mean  $\pm$ SD and is representative of the results obtained from three independent experiments (#p<0.05 compared with the control; \*p<0.05 and \*\*p<0.01 compared with cells cultured with 1 µg/ml LPS).



Figure 3. Inhibitory effects of cynanchone A on LPS-

induced iNOS and COX-2 protein and mRNA expressions in RAW 264.7 cells. Each value indicates the mean  $\pm$  SD and is representative of the results obtained from three independent experiments(#p<0.05 compared with the control; \*p<0.05 and \*\*p<0.01 compared with cells cultured with 1 µg/ml LPS).



#### 3.3. Inhibitory effects of cynanchone a on LPS-induced NF-κB activation and on the nuclear translocations of p50 and p65

To investigate the mechanism responsible for the cynanchone A-mediated inhibitions of the transcriptions of iNOS, COX-2, and TNF- $\alpha$ , we investigated whether cynanchone A regulates LPS-induced NF- $\kappa$ B activity in LPS-stimulated RAW 264.7 macrophages using electrophoretic mobility shift assays (EMSA). As shown in <Figure 4A>, the DNA-binding activity of NF- $\kappa$ B was markedly increased by LPS alone(lane 4), whereas this binding was significantly reduced by cynanchone A pretreatment(lanes 5-7). The specific interaction between DNA and NF- $\kappa$ B was demonstrated by competitive inhibition using excess unlabelled NF-kB oligonucleotides(lane 2). In addition, we also investigated whether cynanchone A prevents the translocations of the p50 and p65 subunits of NF-κB to the nucleus using Western blotting. Negligible levels of nuclei p50 and p65 proteins were detected in control cells, whereas treatment with LPS for 1 h induced the nuclear translocation of both subunits. Western blotting analysis revealed that cynanchone A pretreatment dose-dependently attenuated p50 and p65 levels in nuclear fractions <Figure 4B>. These results suggest that cynanchone A inhibits NF-kB activation by preventing the LPS-induced nuclear translocations of both p50 and p65.

Figure 4. Inhibitory effect of cynanchone A on nuclear translocation of NF-κB. Each value indicates the mean ± SD and is representative of the results obtained from three independent experiments (#p<0.05 compared with the control; \*p<0.05 and \*\*p<0.01 compared with cells cultured with 1 µg/ml LPS).



#### 4. Discussion

Cynanchone A is found in plants, such as, Ocotea suaveloens, Desfontainia spinosa, Hyptis capitata, and Vochysia divergens[15]. Zhang et al. found that low-density lipoprotein receptor-knockout mice fed a cholesterol-rich diet exhibited decreases in atherosclerotic lesion areas of over 50% when they were administered cynanchone A as compared with vehicle treated controls[16]. Furthermore, cynanchone A was reported to suppress in vitro platelet aggregation, particularly when this was induced by epinephrine[17]. In addition, the anti-proliferative and anti-cancer activities of cynanchone A have been shown to be mediated by the inhibition of  $\alpha$  and  $\beta$  DNA polymerases in human gastric cancer cells[12]. Moreover, in a soft agar colony model of TPA-induced skin cancer, cynanchone A treatment reduced tumor growth rates in JB6 cells[18]. Furthermore, previous reports indicate that cynanchone A is non-toxic to normal cells[12]. In murine macrophage RAW 264.7 cells, LPS induces the expression of iNOS, and thus, increases NO production. LPS stimulation is also known to induce I B proteolysis and NF- B nuclear translocation[19]. Therefore, RAW 264.7 cells provide us with an excellent model for drug screening and for subsequently evaluating potential inhibitors of the pathways leading to the induction of iNOS and the production of NO, a major macrophage-derived inflammatory mediator, which has also been reported to be involved in the pathogenesis of many inflammatory diseases[20]. It is well known that macrophages play a crucial role in both non-specific and acquired immune responses. For example, macrophage activation by LPS leads to a functionally diverse series of responses, which include the synthesis and production of NO, prostanoids, and proinflammatory cytokines. In the present study, we evaluated the effects of cynanchone A on the expressions and productions of several pro-inflammatory mediators(iNOS, COX-2, TNF- $\alpha$ ) in LPS-activated macrophages, and found that LPS-induced NO, PGE<sub>2</sub>, and TNF- $\alpha$ dose-deproductions were inhibited pendently by cynanchone A. To explore further the mechanisms underlying these inhibitions, we examined the expressions of iNOS and COX-2 proteins and mRNAs. It was found that cynanchone A inhibited the expressions of iNOS and COX-2 mRNA simultaneously and concentration-dependently <Figure 3>, suggesting that the inhibition of NO and PGE2 release might be attributable to the suppressions of iNOS and COX-2 expression at the mRNA level. It has been reported that TNF- $\alpha$ functions as a pro-inflammatory cytokine in vitro and in vivo[21], and that its production is crucially required for NO synthesis in IFN-y

and/or LPS-stimulated macrophages[22]. Furthermore, TNF- $\alpha$  elicits a number of physiological activities including septic shock, inflammation, cachexia, and cytotoxicity[23]. In the present study, cynanchone A was found to inhibit the expression of TNF- $\alpha$  mRNA <Figure 4>. Of the several transcription factors activated by inflammatory stimuli, NF-KB is known to play critical roles in the expression of pro-inflammatory enzymes and cytokines, such as, iNOS, COX-2, and TNF- $\alpha$ [24]. In the present study, we examined the possibility that cynanchone A inhibits NF-kB activity in vitro, and found that cynanchone A effectively prevented LPS-inducible NF-kB-DNA binding activity. In addition, we found that cynanchone A dose-dependently inhibited the translocation of activated NF-KB to the nucleus and the degradation and phosphorylation of  $I\kappa B-\alpha$ .

#### **5.** Conclusion

In conclusion, our results demonstrate that cynanchone A exerts anti-inflammatory effects by inhibiting NF- $\kappa$ B activation in macrophages, and thus, prevents the expressions of iNOS, COX-2, and TNF- $\alpha$ . Accordingly, our results suggest that cynanchone A may have a potential agent for inflammatory disease.

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## Effects of Betting Elements on Self-Management of Amateur Golfers Paper

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#### Abstract

This study aimed to investigate the effects of the golf-addiction tendencies of golf betting participants on their self-management practices. About 450 survey questionnaires(data sheets) were completed by and collected from adults aged 20 years and above with at least 3 years of golfing experience who reside in the Seoul and Gyeongin area in South Korea. The data were analyzed, and the results revealed that, depending on the golfers' history of betting participation, there were significant differences in golf addiction tendencies and self-management behavior between the two participant groups(betting vs. non-betting). Across all factors relating to golf addiction, the tendencies of wagering participants were found to be higher than those of non-participants. In terms of training-related self-management behavior, betting participants still showed higher tendencies than non-betting golfers, indicating that wagering in golfing could promote competitiveness in the game and increase the players' dependence on golfing. Compared with other sports, golf can be intensely addictive, and, thus, golfing might be subject to gambling-like factors. Future studies should clarify the difference between wagering and gambling and, in particular, between golf betting and golf gambling. Follow-up studies could further investigate the proper, socially acceptable levels of betting.

[Keywords] Golf, Gambling, Addiction, Wagering, Self-Management

#### 1. Introduction

Of all sports, golf may be the most unique. Golf entails some challenges, such as making a swing(an unnatural act in and of itself) more natural, as well as variables that can lead to varying degrees of fun and results(scores). Most sports/games have elements of competition. Golf, however, embodies not only a competitive characteristic but a degree of gambling as well, which makes the sport extremely peculiar. Many people who enjoy golf put various efforts into improving their performance. On the flip side of such dedication, a considerable measure of motivation is expected to be inspired when, frequently, golfers want to win a bet(wagering) while engaging in rounds with other players. Although

many golfers enjoy their rounds for the purpose of promoting their health or social relationships with others, it is equally true that countless numbers of rounds are played with the aim to wager money(golf bet). Moreover, it would not be an overstatement to say that such golf betting is the core characteristic of Korean amateur golfing culture[1]. Any golfer may engage in golf bets at least once, which, in most cases, take the form of paying for the winners' meals or gratuity for caddies[2]. Wagering has been reported to help improve performance by increasing the entertainment element of games, enhancing the concentration during rounds, and motivating golfers to practice more[3]. Despite the merits, golf bets also have extremely negative characteristics in that players participate not for sportsmanship but solely to win the money,

thereby altering the winning spirit to one of gambling. Golf bets are addictive in nature, just like doing drugs, drinking, Internet activities, gambling, and so on. Cho Hyun-ik, Kim Yong-jun, and So Yeong-ho(2006)[4] reported that, the more the nature of golf tournaments resembles gambling, the more the tendency of golf addiction increases in players because of the intense excitation and emotional gratification that gambling can offer. The findings indicate that, for those participating in golf as a form of gambling, such participation goes beyond wholesome aims, such as promoting health, enjoying leisure activities, and pursuing hobbies, and enters the realm of gambling, which bears a strong additive appeal. That said, unlike typical characteristics shown in gambling addicts, golf betting addiction tendency can be seen as a positive kind of addiction in that the drive helps individuals increase their psychological and physical vitality and improve their well-being and physical functioning, thereby complementing daily living[5]. In some studies, however, the phenomenon of gambling tendencies manifested to a certain degree in golfing might spread to individual as well social problems[6][7][8][9]. Hence, the addictive nature found in gambling-oriented golfing has the same characteristics as typical gambling activities and is thus considered a variable that has an important bearing on golf-bet participation[4]. If appropriate, the betting element in golfing could increase the level of focus and fun in players and could help improve their concentration during long hours of rounds through appropriate levels of tension. Many researchers report that self-management skills are essential for golfers, because they help the players control or adjust their emotions and maintain calm to decrease their scores while adjusting to the various discrepancies of clubs, other players, and the milieu for many hours at one stretch[10]. Given the demands, golfers' self-management is extremely important, and good performance on rounds requires efforts on the part of players. Furthermore, self-management helps golfers accomplish a match between their intentions and goals, which will bring about far better scores, helping to improve individual players' golfing techniques and emotional skills exceeding

those of their opponents, helping to maintain concentration for a long time. Orlick and Partington(1988)[11] reported that, when comparing outstanding golfers to those not performing well, the former were found to extremely excel in focus for success, effective training skills based on established goals, emotional preparation for simulation games, extremely meticulous tournament-preparing strategies, and contingency planning against uncertain situations during tournaments. Previous studies[1][4][12][13][14][15][16] mostly regard golf bets as a gambling activity, thus stressing only the negative aspects. Hence, it was difficult for the present research to locate an objective perspective on the emotional effects of wagering during golf. The present study identified a necessity to research the roles of betting as a leisure activity during rounds, analyze the emotional effects, and objectively understand golfing and golf bets. Accordingly, this study compared and analyzed the exercise addiction tendencies found in golf-bet participants and non-participants, respectively, and aimed to explain the addictive tendencies in golf bets and the relationships between betting(wagering) in leisurely pursued golfing and the sub-factors for self-management.

#### 2. Measurement Tools

The research tool used for this study was a series of structured survey questionnaires. The questions were roughly divided into demographic-statistical characteristics, selfmanagement, exercise addiction, and leisure satisfaction. The validity of the questions was already verified through previous studies. Specifically, the questionnaire consists of 3 questions for personal characteristics, 23 for self-management, 37 for exercise addiction, and 21 for leisure satisfaction. For each question item, a 5-point Likert rating scale was utilized. For measuring the validity and reliability for each measuring tool, exploratory factor and Cronbach's  $\alpha$  analyses were performed. Exploratory factor analysis used principle component analysis as the factor extraction model, varimax rotation was utilized as the rotation method, and only those factors with eigenvalue 1.0 or above were extracted.

#### 2.1. Betting and golf addiction

The questionnaire used for this study regarding golf betting and golf addiction was a modified and complemented version of the Korean-style betting and exercise addiction questionnaire developed by Cho Yong-gyu (2007)[17] in consideration of the study purpose. The questionnaire consists of a total of 35 guestions and 5 factors. For exercise addiction type analysis, factor analysis was conducted, identifying 5 constituting factors. Factor 1 refers to emotional attachment, Factor 2 to lack of control, Factor 3 to withdrawal, Factor 4 to golf urge, and Factor 5 to golf dependence. For reliability tests, the results were .917 for emotional attachment, .823 for lack of control, .834 for withdrawal, .775 for golf urge, and .735 for golf dependence.

#### 2.2. Self-management

The self-management questionnaire used for this study was based on one designed by Hur Jeong-hun (2002)[18] for athletes' selfmanagement comprising a total of 25 items and 4 factors. The Hur version was modified and complemented for the purpose of this study. The sub-factors for self-management were physical care, mental care, training and management, and interpersonal management. For self-management type analysis, factor analysis was designed with 4 factors. Factors 1 through 4 were designated as mental care, interpersonal management, physical care, and training and management, in that order. Results of reliability were .775(F 1), .736(F 2), .683(F 3), and .644(F 4), in the same order.

#### 2.3. Data processing

Conducting this study, the data sheets containing the participants' responses to the questionnaire items were collected and reviewed for screening purposes. Any data sheets with incomplete or unreliable responses were excluded from analysis, as were any outliers. The input data were then processed using SPSS WIN 18.0 for the purpose of data analysis. The statistical methods adopted for data analysis were reliability and factor analyses. Furthermore, t-test was carried out to verify the difference. Correlation and multiple regression analyses were performed to examine relationships between variables, in particular stepwise regression. For all significance levels,  $\alpha$  was set at <.05.

### 3. Results

#### 3.1. Golf addiction depending on golf-betting participation

Results of the verification of differences in golf addiction depending on the participation history of golfers who engage in wagering were found to be statistically significant for all factors. Across factors, the differences were all larger in golf-betting participants with wagering participants showing higher levels of emotional attachment while nonparticipants showed the highest levels of golf urge. All of the mentioned participants showed the lowest levels for lack of control.

#### 3.2. Golf addiction depending on golf-betting participation

According to the results of the verification of differences in self-management depending on the participants' golf-betting engagement history, significant statistical differences were found in interpersonal management(t = -1.170, p < .05) and training and management(t = 1.141, p < .05). The former had a higher percentage of golfers who did not participate in wagering whereas training and management had a higher percentage of betters than non-betters.

#### 3.3. Relationship between golf addiction and self-management

Results of verification of betting participants' golf addiction and self-management factors showed that, for golf addiction, there were statistically significant positive correlations between emotional attachment and golf dependence, golf urge, withdrawal, training, lack of control, social leisure, environmental psychology, mental care, physiology and physical, physical care, and interpersonal management, in that order. Significant positive correlations were found between lack of control and withdrawal, golf dependence, golf urge, and social leisure, in that order. There was significant negative correlation with interpersonal relationship and significant positive correlations between withdrawal and golf dependence, golf urge, social leisure, physiology and physical, environmental psychology, and training, in that order. For golf urge, significant positive correlations were found with social leisure, environmental psychology, physiology and physical, mental care, golf dependence, interpersonal management, and training, in that order; significant positive correlation was found between golf dependence and training. For self-management, there were statistically significant positive correlations found with mental care in the order of interpersonal management, physiology and physical, environmental psychology, social leisure, training, and physical care; significant positive correlations were found with interpersonal management in the order of physiology and physical, environmental psychology, social leisure, and training. With physical care, significant positive correlations were found in the order of environmental psychology, physiology and physical, social leisure, and training, and, with training, significant positive correlations were found in the order of physiology and physical, social leisure, and environmental psychology.

# **3.4.** Effects of golf addiction on self-management with non-betting golfers

According to the results of analysis on the effects of non-betting participants' golf addiction on their self-management, in terms of the non-participants' self-management subfactor mental care, lack of control( $\beta = -.424$ ), emotional attachment( $\beta = .489$ ), and withdrawal( $\beta = -.211$ ) accounted for about 35.7% of the total mental-care variance; for interpersonal management, golf urge( $\beta = .146$ ), lack of control( $\beta = -.250$ ), and golf dependence( $\beta = .259$ ) together accounted for about 15.7% of the total interpersonal-management variance; for physical care, withdrawal ( $\beta = -.378$ ), emotional attachment( $\beta = .269$ ), and golf urge( $\beta = -.274$ ) together accounted

for approximately 28.5% of the total physicalcare variance; and, for training and management, emotional attachment( $\beta$  = .476), lack of control( $\beta$  = -.375), and golf dependence( $\beta$ = .293) together accounted for around 38.0% for the total training-and-management variance.

#### 4. Discussion

The purpose of this study was to investigate the effects of the characteristics of golfbetting behavior on the players' golf addiction tendencies and self-management. Depending on the players' history of participating in golf betting (yes vs. no), for all factors, golf addiction was found to be higher in betting participants than in non-participants. The finding matched the results reported by the study of Choi Han-bin (2009)[19], in which players of competitive sports showed higher dependence tendencies for exercise addiction than players of conquest sports. In the study by Yu Gi-seong(2007)[20], the same results were found that those playing sports that require win-lose outcomes, competition, or concentration for victory exhibited strong exercise-addiction behavior. Of note, the stronger the tenacity for winning, the stronger the dependent addiction tendencies; hence, golf betting could have increased the competitive element of golf tournaments against the leisure activity characteristic, turning golf into a more competitive sporttype activity. Compared with other sports, golf could take on a particularly strong aspect of competitiveness due to frequent betting practices, and the betting participants could have shown higher tendencies than did the non-participants. Furthermore, in terms of the self-management factor and, in particular, the training and management factor, wagering participants showed higher tendencies than non-participants. The finding is similar to that reported by Sohn Seok-jeong found (2005)[3],who betting helps strengthen competition and improve performance, and at least partially similar to the results of the study by Hong Hee-seon (2009)[21], in which, the better the players, the higher their self-management tendencies. The foregoing could be similar to the research findings reported by Rosenthal(1992)[22], who discovered that, in cases of minor leisure-minded gambling, participants could, regardless of winning or losing, enjoy themselves without overindulging in the gambling or accompanying dysfunction. According to the results of golfers' golf addiction and selfmanagement, emotional attachment(golf addiction) and golf urge(golf addiction) were found to have a partial correlation with selfmanagement; lack of control(golf addiction) showed a negative correlation with self-management; and, especially for the interpersonal factor, a direct correlation was found. The finding of this study—i.e., the stronger the golf addiction tendency, the greater focus the players place on their training-shares some similarities with the findings of Choi Han-bin(2009)[19], who reported that a continued participation in sports did have direct influences on exercise addiction. However, exercise addiction is like two sides of the same coin, having both positive and negative effects. Hence, researchers who study the exercise addiction phenomenon should not ignore the fact that the addictive behavior found in golf betting participants might be positive at first but difficult to control and, if taken to the extreme, could show compulsive behavior patterns controlled by unpleasant pressure that is against one's own willpower [8]. In sum, for betting elements to offer some measure of emotional gratification and positive sports-activity experience in golf participants, an appropriate level of wagering is necessary.

#### **5. Conclusion and Suggestions**

The purpose of this study was to investigate the effects of golf-betting participants' golf addiction on their self-management and leisure satisfaction. To that end, the study recruited adults aged 20 years and above from the Seoul and Gyeongin area and analyzed 450 survey questionnaires(data sheets) and obtained the following results. First, depending on the golf players' golf betting history, there were differences between golf addiction and self-management. Across all factors

relating to golf addiction, the tendencies of betting participants were found to be higher than those for non-participants, indicating that betting further strengthened the competitive nature of golf rounds, thus increasing the players' dependence on golf. Among the self-management-related factors, training and management showed higher tendencies in betting participants than in non-participants, and wagering was found to help improve the players' performance and increase their sense of immersion. The golf-addicted tendencies in wagering participants were found to have positive effects on the participants' self-management, while in non-participants, the same tendencies at least partially influenced the non-participants' self-management. The study drew a conclusion that the psychological effects of golf betting on the players' behavior during rounds are manifested in many aspects. It would be necessary for future studies to offer proper academic definitions of wagering(betting) and gambling. Regarding golf betting(gamblinglike golfing), vagueness in concepts still prevails; hence, golf betting itself is viewed by some as ethically negative. Future research should clarify the concept of wagering vs. gambling as well as golf betting vs. golf gambling. Furthermore, it would be necessary for studies to investigate various psychological effects of golf betting and further systematize the characteristics of betting.

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