行政院國家科學委員會專題研究計畫 期末報告

探討馬蹄蛤之蛋白質組成、抗氧化活性、保肝作用和 PCR-RFLP 鑑種技術之建立(第 3 年)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 99-2313-B-040-006-MY3
執 行 期 間 ：101 年 08 月 01 日至 102 年 07 月 31 日
執 行 單 位 ：中山醫學大學健康餐飲暨產業管理學系（所）

計 畫 主 持 人：葉彥宏
共 同 主 持 人：謝宥諒、謝宥諒、謝宥諒
計畫參與人員：碩士班研究生-兼任助理人員：楊惠鈞

公 開 資 訊 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 102 年 10 月 30 日

中 文 摘 要 ： We investigated the effect of Geloina eros extract against carbon tetrachloride (CCl4)-induced hepatotoxicity.
Our results showed that treatment with Geloina eros extract for 8 weeks significantly reduced the impact of
CCl4 toxicity on the serum markers of liver damage, aspartate aminotransferase (AST), alanine aminotransferase
(ALT) and alkaline phosphatase (ALP) Antioxidant system was significantly enhanced in the plasma, and hepatic thiobarbituric acid reactive substance (TBARS) levels were lowered while the hepatic superoxide dismutase
(SOD) and catalase (CAT) activities and glutathione peroxidase (GSH-Px) protein level and nonenzymatic antioxidants (vitamin E, vitamin C and GSH) were elevated. The results indicated that Geloina
eros extract has a protective effect against acute hepatotoxicity induced by the administration of CCl4 and
was found to be comparable to that of silymarin and have been supported by the evaluation of the liver histopathology
in rats. The hepatoprotective effects of Geloina eros extract may be due to both the inhibition of
lipid peroxidation and the increase of antioxidant activity.

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附件一

行政院國家科學委員會補助專題研究計畫 □期中進度報告 ■期末報告

探討馬蹄蛤之蛋白質組成、抗氧化活性、保肝作用和 PCR-RFLP 鑑種技術之建立

計畫類別：■個別型計畫　□整合型計畫
計畫編號：NSC 99－2313－B－040－006－MY3
執行期間：99 年 8 月 1 日至 102 年 7 月 31 日

執行機構及系所：中山醫學大學 健康餐飲管理學系

計畫主持人：葉彥宏
共同主持人：謝宥諒

本計畫除繳交成果報告外，另須繳交以下出國報告：
□赴國外移地研究心得報告
□赴大陸地區移地研究心得報告
□出席國際學術會議心得報告及發表之論文
□國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年■二年後可公開查詢

中　華　民　國　102　年　10　月　28　日

附件二

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■ 達成目標

□ 未達成目標（請說明，以 100 字為限）

□ 實驗失敗

□ 因故實驗中斷

□ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

To investigate the effect of *Geloina eros* extract against carbon tetrachloride (CCl_4)-induced hepatotoxicity. Our results showed treatment with *Geloina eros* extract for 8-weeks significantly reduced the impact of CCl_4 toxicity on the serum markers of liver damage, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and antioxidant system was significantly enhanced in the plasma and hepatic thiobarbituric acid reactive substances (TBARS) levels were lowered while the hepatic superoxide dismutase (SOD) and catalase (CAT) activities and glutathione peroxidase (GSH-Px) protein level and non-enzymatic antioxidants (vitamin E, vitamin C and GSH) were elevated. The results indicated that *Geloina eros* extract has a protective effect against acute hepatotoxicity induced by the administration of CCl_4 was found to be comparable to that of silymarin and have been supported by the evaluation of the liver histopathology in rats. The hepatoprotective effects of *Geloina eros* extract may be due to both the inhibition of lipid peroxidation and the increase of antioxidant activity.

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Protective effects of *Geloina eros* extract against carbon tetrachloride-induced hepatotoxicity in rats

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ARTICLE INFO

ABSTRACT

1. Introduction

Among the various health problems suffered by people in Taiwan, liver diseases including hepatocellular carcinoma, fibrosis, cirrhosis and hepatitis appear to be one of the most serious (Wu, Danielsson, & Zern, 1999). Hepatotoxins, such as ethanol, acetaminophen, and carbon tetrachloride (CCl_4), sparked off liver injury which it is characterized by varying degrees of hepatocyte degeneration and cell death (Wu, Danielsson, & Zern, 1999). Vitaglione et al. (2004) suggested that reactive oxygen species (ROS) including superoxide and hydroxyl radicals are known to play an important role in liver disease's pathology and progression as well as ROS have been proved to associate with the intoxication by CCl_4 (Slater & Sawyer, 1971). Documented evidences suggested that CCl_4 has been commonly used as hepatotoxins in experimental hepatopathy (Hsu et al., 2008). Covalent binding of the metabolites of CCl_4, trichloromethyl free radicals, to cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell death (Recknagel et al., 1989). Many studies reported that natural antioxidants are efficacious to prevent oxidative stress-related liver pathologies due to particular interactions and synergisms (Bhathal et al., 1983; Vitaglione et al., 2004; Cheng et al., 2011). ROS production is linked with oxidative stress which is defined as the imbalance in the generation of oxidants and the antioxidant defense (Cogger et al., 2004; Upur et al., 2009). Regarding the central role of ROS in liver disease and pathology, antioxidants might prevent hepatic damage through scavenger activity and increase the activity of intracellular antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). There are a number of evidences indicating that natural substances from edible and medicinal plants exhibited strong antioxidant activity that could act against hepatic toxicity caused by various toxicants (Upur, Amat et al., 2009; Harish & Shivanandappa, 2006; Zeashan et al., 2008; Madureira et al., 2011).

A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH-Px, which convert active oxygen molecules into non-toxic compounds. One of such candidates is *Geloina eros* extract, which was chosen in the present study.

Hard calm, oyster and abalone are popular seafood and traditionally used as a Chinese remedy for liver disease and chronic hepatitis in the folk medicine. Many researchers have revealed that marine shellfish extracts have biological properties, such as antitumor and antihepatitis (Pan et al., 2006; Pan et al., 2007). Recently, the specie clam of *Geloina eros* (the shell length, width and height of clams range from 4.96~5.77 cm, 2.82~2.92 cm and 5.27~5.64 cm) is the largest indigenous were to cultivate in Yulin County of Taiwan, however, there exist no research reported on *Geloina eros*. Therefore, in the present study, we investigated the activity of *Geloina eros* extract against CCl_4-induced oxidative stress and hepatotoxicity in the rats for 8 weeks, hepatic GSH-Px and thiobarbituric acid reactive substances (TBARS) levels as well as activities of AST, ALT and ALP in serum and CAT, SOD and GSH-Px in liver tissues were measured to monitor liver injury. The extent of CCl_4-induced liver injury was also analyzed through histopathological examination.

2. Materials and methods

2.1. Materials

Carbon tetrachloride (CCl_4), olive oil and silymarin were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals used were in the purest form available commercially.

2.2. Preparation of Geloina eros extract

Geloina eros was collected from Zeng Jiechong aquaculture farm (Yulin, Taiwan), tissue from fresh *Geloina eros* (5,000 g) was cut into small pieces, homogenized in a blender and extracted with 5.0 L ethyl acetate. This procedure was repeated three times. The resulting supernatant extract was filtered and concentrated by rotary evaporator working under a vacuum and then freeze-dried. The yield of *Geloina eros* powder was 10.0 g for the experiments was stored at -18℃.

2.3. Animals

Male Wistar rats, weighing 210-220 *g*, were purchased from National Laboratory Animal Center and housed individually in stainless steel wire bottom cages with a control environment (25℃, 50-60% humidity, 12 *hr* light per day) for two weeks acclimatisation. The animals were fed a laboratory diet (PMI Feeds, USA). Tap water was supplied in free access. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for in accordance with the institutional ethical guideline.

2.4. Treatment

Two weeks later, the animals were randomly divided into seven groups with each consisting of 8 rats. Group A basal diet (without the addition of *Geloina eros* extract with a formulation based on American Institute of Nutrition (1977) (AIN-93M diet) for a period of 8 weeks, Group B basal diet (with the addition of *Geloina eros* extract) 6% at doses of 0.1 g/kg body weight for a period of 8 weeks. For inducing hepatotoxicity, animals of groups C–F were given carbon tetrachloride at a dose of 0.1 ml/100 g body weight of CCl_4, orally, (20% v/v in olive oil) twice a week for a period of 8 weeks of Group A and B received olive oil and saline served as vehicle control animals. After CCl_4 intoxication, Group C served as control CCl_4. Groups D–F were administered *Geloina eros* extract in diet for 6% at doses of 100 mg/kg, 7% at doses of 116.69 mg/kg, 8% at doses of 133.36 mg/kg, Group G served as positive control and was given silymarin in diet for 12% at doses of 200 mg/kg, respectively, daily for a period of 8 weeks. All other groups were fed the basal diet, its components are listed in Table 1. On weeks 2 and 4, blood was obtained by tail vein puncture 6 hrs after administration. On week 8, the rats were weighed and anesthetized with diethyl ether. Blood was obtained by heart puncture with syringes.

Plasma was collected by centrifugation (1,000 *g* ×15 *min*) from blood and analyzed using a Merck VITALAB Selectra Biochemical Autoanalyzer (Merck, Germany) to determine blood urea nitrogen (BUN), creatinine, aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP). Livers and kidneys of the rats were quickly excised and weighed. Both relative ratios of liver and kidney weight to body weight were obtained. The liver was stored at -40℃ for glutathione peroxidase (GSH-Px) and thiobarbituric acid reactive substances (TBARS) determinations.

2.4. Antioxidant activities

Appropriate liver tissues were dissected, weighed, immersed in liquid N_2 within 60 s of death, and kept frozen at -70℃. Prior to enzyme determinations, thawed tissue samples were homogenized in 20 volumes of ice cold 50 mM phosphate buffer (pH 7.4), centrifuged at 3200×3 g for 20 min at 5℃. The supernatant was used for antioxidant enzyme determinations.

(I) *CAT activity*

The catalase (CAT) activity was measured using Aebi's (1974) method with a slight modification. The mitochondria pellet was dissolved in 1.0 mL of a 0.25 M sucrose buffer. Ten μL of the mitochondria solution was added to a cuvette containing 2.89 mL of a 50 mM potassium phosphate buffer (pH 7.4), then the reaction was initiated by adding 0.1 mL of 30 mM H_2O_2 to make a final volume of 3.0 mL at 25℃. The decomposition rate of H_2O_2 was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to determine the CAT activity. The activity was defined as the μmol H_2O_2 decrease/mg protein/min.

(Ⅱ) *SOD activity assays*

The superoxide dismutase (SOD) activity was measured using Marklund & Marklund (1974) method with a slight modification. One hundred uL of the cytosol supernatant was mixed with 1.5 mL of a Tris-EDTA-HCl buffer (pH 8.5) and 100 μL of 15 mM pyrogallol, and then incubated at 25ºC for 10 min. The reaction was terminated by adding 50 μL of 1N HCl, then the activity was measured at 440 nm. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as units/mg protein.

(Ⅲ) *Levels of GSH-Px measurement*

Glutathione peroxidase (GSH-Px) levels were measured using the glutathione assay kit (Calbiochem, San

Diego, CA). An equal volume of ice cold 10% metaphosphoric acid was added to the liver preparations. Supernatants were collected after centrifugation at 1000 rpm for 10 min and analysed for GSH-Px as per manufacturer's instructions. Total GSH-Px in the samples was normalised with protein (Paglia & Valentine, 1967).

(Ⅳ) *Levels of GSH measurement*

GSH of glutathione reacts non-enzymatically with 5,5'dithiobis (2-nitrobenzoic acid) (DTNB) to yield glutathione disulfide (GSSG) and 2-nitro- 5-thiobenzoic acid (TNB). GSSG is then reduced enzymatically by NADPH and glutathione reductase (GR) to regenerate GSH. Concentrations of DNTB, NADPH and GR are chosen such that the rate of the overall reaction is linearly proportional to the concentration of total glutathione. The rate of formation of TNB is followed spectrophotometrically, and assay is calibrated using standards. If the sample is reacted with 2-vinylpyridine, GSH is derivatized, and only GSSG is detected during subsequent assay (Griffith 1980).

(V) *TBARS concentration*

Lipid peroxidation activities in the liver and plasma were assayed by measurement of MDA, an end-product of peroxidized fatty acids, and TBA reaction product. The sample of 20% liver homogenate was mixed with 1.0 ml 0.4% TBA in 0.2 N HCl and 0.15 ml 0.2% BHT in 95% ethanol. The samples were incubated in a 90℃ water-bath for 45 min. After incubation, the TBA-MDA adduct was extracted with isobutanol. The isobutanol extract was mixed with methanol (2:1) prior to injection into the system of HPLC. The supernatant was examined by using the HPLC system at an excitation 515 nm and an emission 550 nm on a Hitachi Fluorescence Detector (Japan) (Tatum, Changchit, & Chow, 1990).

(VI) *Levels of ascorbic acid measurement*

Ascorbic acid (vitamin C) concentration was measured by Omaye et al. (1979) method. To 0.5 ml of sample, 1.5 ml of 6% TCA was added and to 0.5 ml of DNPH reagent (2% DNPH and 4% thiourea in 9 N sulfuric acid) mixed with and incubated for 3 h at room temperature. After incubation, 2.5 ml of 85% sulfuric acid and colour developed was read at 530 nm after 30 min.

(VII) *Levels of vitamin E measurement*

Vitamin E estimated by the method of Desai (1984). Lipid extract was prepared by Folch et al. (1957). To 0.5 ml of lipid extract, 1.5 ml ethanol, 2.0 ml of petroleum ether were added and centrifuged. The supernatant was evaporated to dryness at 80℃, to that added 0.2 ml of 2,20-dipyridyl (2%) and, 0.2 ml of ferric chloride (0.5%), kept in dark for 5 min and then 4 ml butanol was added. The colour developed was read at 520 nm.

2.5. Histopathological examination

The livers were preserved in 10% buffered formalin for at least 24 h, dehydrated with a sequence of ethanol solutions and were processed for embedding in paraffin. Sections of 5–6 mm in thickness were cut, deparaffinized, rehydrated, stained with haematoxylin and eosin (H&E) for the estimation of hepatocyte necrosis and vacuolization, as well as Masson trichrome stain and Sirius red stain for hepatocyte fibrosis, were subjected to under the photomicroscopic examination. The histological scoring of hepatic damage and fibrosis were expressed using the following score system: 0, no histopathologic change; 1≤, mild histopathologic change; 2≤, moderate histopathologic change; 3≤, Severe histopathologic change (Kondou et al., 2003).

2.6. Statistical analysis

All data were analyzed by one-way ANOVA (Puri & Mullen, 1980). Duncan's new multiple range tests was used to revolve the difference among treatment means. All statistical analyses were performed using the statistical software SPSS 11.0 (SPSS Ltd., Surrey, UK). A P value<0.05 was considered statistically significant. Ratio values were not arcsin transformed before statistical analysis.

3. Results

3.1 Body weight, liver and kidney weight to body weight

The effects of CCl_4 and *Geloina eros* extract on the growth of rats are shown in Table 2. After 8-week feeding, the weight of the rat was significantly decreased (P<0.05) when in the hepatotoxic model group (Group C), but significantly increased (P<0.05) when the diet was supplemented with *Geloina eros* extract in CCl_4 treated groups (Group D-G). The effects of CCl_4 and *Geloina eros* extract on the ratios of liver and kidney weight to body weight in rats are shown in Table 2. After 8-week feeding, the ratios of liver and kidney weight to body weight of rats in the hepatotoxic model group (Group C) were more significantly

decreased compared to those of rats fed with the control diet and *Geloina eros* extract diet. However, the ratios of liver and kidney weight to body weight in rats fed diet supplemented with CCl_4 and *Geloina eros* extract were significantly different from those of rats fed diet supplemented with *Geloina eros* extract. This means that *Geloina eros* extract might significantly reduce the toxicity of CCl_4 in the rats based on the ratios of liver and kidney weight to body weight.

3.2 Effect of *Geloina eros* extract on CCl_4-induced liver injury in rats

The effect of various doses of *Geloina eros* extract on serum biochemical markers in CCl_4-intoxicated rats was studied (Fig. 1). After a single injection of CCl_4, serum activities of AST, ALT and ALP enzymes in the CCl_4 treated groups (Group C-G) were significantly increased ($P<0.05$), as compared to the normal control group (Group A), respectively. Treatment of animals with different doses of *Geloina eros* extract for 8 weeks significantly reduced the activities of serum AST, ALT and ALP as compared to the group of CCl_4-treated alone. Positive control drug, silymarin, at dose of 200 mg/kg also reduced the levels of serum AST, ALT and ALP.

3.3 Effects of rat kidney

There was no significant difference ($P>0.05$) in the concentration on BUN and creatinine in the plasma among various groups.

3.4 Effects of *Geloina eros* extract on non-enzymatic antioxidants

Fig. 2 represents the levels of non-enzymatic antioxidants (vitamin E, vitamin C and GSH) status in tissues. The levels of vitamin E, vitamin C and GSH were significantly ($P<0.05$) reduced in CCl_4-treated rats when compared with control rats. Administration of *Geloina eros* extract (Group D-F) and silymarin significantly ($P<0.05$) restored the levels of non-enzymatic antioxidants in tissues.

3.5 Effects of *Geloina eros* extract on antioxidant enzymes

The hepatic antioxidant enzyme activities of SOD and CAT were decreased in the liver of rats treated with CCl_4, however, activity of SOD and CAT was restored by *Geloina eros* extract (Group D-F). As shown in Fig. 3, the hepatic GSH-Px level was markedly lower in CCl_4-intoxicated rats. However, the GSH-Px level was significantly increased by *Geloina eros* extract (Group D-F) treatment when compared with the CCl_4 group. Administration of silymarin (Group G) did not significantly increase ($P>0.05$) the activities of SOD, CAT and GSH-Px respectively, as compared to the CCl_4-treated group. Expected increases of the hepatic and serum lipid peroxidative indices in the CCl_4-treated model group confirmed that oxidative damage has been induced (Fig. 3), treatment with various doses of *Geloina eros* extract (Group D-F), the levels of TBARS in the liver and plasma were significantly lower than those in the CCl_4-treated model group. Silymarin (Group G) also inhibited the elevating TBARS levels upon CCl_4 administration.

3.6 Histopathologic examination

The histological observations supported the results obtained from serum enzymes assays. Liver sections from control rats showed normal lobular architecture and hepatic cells with a well-preserved cytoplasm and well-defined nucleus and nucleoli (Fig. 5). The results of hepatic histopathological examination are shown in Table 3. When compared with the normal liver tissues of vehicle controls, liver tissue in the rats treated with CCl_4 revealed extensive liver injuries characterised by moderate to severe hepatocellular hydropic degeneration and necrosis around the central vein, lipidosis, hepatic fibrosis and cholangiocyte hyperplasia. However, the histopathological hepatic lesions induced by administration of CCl_4 were only remarkably ameliorated in central lobular necrosis, hepatic lipidosis, and hepatic fibrosis by treatment with *Geloina eros* extract and silymarin.

4. Discussion

In the present study, the capability of *Geloina eros* extract to protect against CCl_4-induced hepatotoxicity and oxidative stress was investigated. CCl_4 is a mild analgesic and antipyretic agent (Recknagel, 1967; Recknagel et al., 1989). It is also known that its oxy metabolite can produce significant hepatic toxicity through the depletion of GSH-Px level in the liver. This active metabolite reacts with liver to induce lipid peroxidation and causes hepatic cell death resulting in an elevated of serum enzyme AST, ALT and ALP (Weber, Boll, & Stampfl, 2003).

We studied further other underlying mechanisms responsible for this hepato-protective action of *Geloina eros* extract in the animal. The protective effect of *Geloina eros* extract was accompanied with a partial prevention of GSH-Px depletion in the liver tissue. It is considered that hepatic GSH-Px represents is an enzyme reserve of the liver, which is responsible in reducing the hepatotoxicity induced by the active metabolites of CCl_4. As GSH-Px is also a crucial determinant of tissue susceptibility to oxidative damage

(Ischiropoulos et al., 1992), the partial protection of *Geloina eros* extract on GSH-Px reserves provide an additional action, not only to remove the active metabolites of CCl_4, but also to scavenge free radicals, which are involved in lipid peroxidation.

CCl_4 also increased lipid peroxidation, and as a result the hepatic TBARS level was elevated (Szymonik-Lesiuk et al., 2003). *Geloina eros* extract treatment prevented this effect, indicating that *Geloina eros* extract was able to attenuate the lipid peroxidation induced by CCl_4.

Hepatocellular necrosis leads to elevations of serum AST, ALT and ALP activities and an increased incidence and severity of histopathological hepatic lesions in rats. The present study revealed a significant increase in the activities of AST, ALT and ALP on exposure to CCl_4 indicating considerable hepatocellular injury. Administration of *Geloina eros* extract attenuated the increased levels of the serum enzymes (AST, ALT and ALP) induced by CCl_4 and caused a subsequent recovery towards normalization comparable to the control group and the good hepatoprotective effects is comparable with silymarin. The effect of *Geloina eros* extract was further confirmed by the histopathological examinations. *Geloina eros* extract offers hepatoprotection effective in central lobular necrosis, hepatic lipidosis, and cholangiocyte hyperplasia than other lower doses in rats. It has been hypothesised that one of the principal causes of CCl_4-induced liver injury is formation of lipid peroxides by free radical derivatives of CCl_4. Thus, the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4-induced hepatopathy. The body has an effective defense mechanism to prevent and neutralize the free radical induced damage. This is proficient by a set of endogenous antioxidant enzymes such as SOD and catalase. These enzymes constitute a mutually supportive team of defense against reactive oxygen species (ROS) (Vitaglione et al., 2004). Lipid peroxidation, a ROS-mediated mechanism, has been implicated in the pathogenesis of various liver injuries and subsequent liver fibrogenesis in experimental animals (Niemela et al., 1994). The significant non dose-dependent (100-133.36 mg/kg body weight) decrease in the hepatic lipid hydroperoxide confirmed that treatment with *Geloina eros* extract could effectively protect against the hepatic lipid peroxidation induced by CCl_4. Hence, it is possible that the mechanism of hepatoprotection of *Geloina eros* extract may be due to its antioxidant activity.

Non-enzymatic antioxidants such as GSH, vitamin C and vitamin E are closely interlinked to each other and play an excellent role in protecting the cell from lipid peroxidation. The depleted level of GSH in CCl_4-treated may due to scavenging of toxic radicals and inhibition of the synthesis and increased rates of turnover (Kuo et al., 2010). In addition to GSH, we have also observed a decrease in the levels of antioxidants such as vitamin C and E in tissue of CCl_4-treated rats. Supplementation of *Geloina eros* extract to CCl_4-treated rats restored the non-enzymatic antioxidants levels in liver. The biologically active antioxidants found in *Geloina eros* extract sparing the antioxidant activity and reduced the consumption of endogenous antioxidants, which could be responsible for the reduction of oxidative stress during CCl_4 toxicity. The animal groups treated with *Geloina eros* extract showed an increase in the level of SOD and CAT, which indicated the antioxidant activity of the *Geloina eros* extract. GSH-Px acts as a enzymatic antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes that reduces hydrogen peroxide and hydroperoxides. The depletion of hepatic GSH-Px has been shown to be associated with an enhanced toxicity to chemicals, including CCl_4 (Alı´a et al., 2003). In the present study, a decrease in hepatic tissue GSH-Px level was observed in the CCl_4-treated groups. The increase in hepatic GSH-Px levels in the rats treated with *Geloina eros* extract may be due to de novo GSH-Px synthesis or GSH-Px regeneration.

In the current study, treatment with the *Geloina eros* extract partially prevented the TBARS in liver and plasma. This action could alleviate the injurious action of the oxidative stress on the liver. Further studies with individual active compounds isolated from *Geloina eros* extract on hepatocytes are underway which will enable us to understand the exact mechanism of hepatoprotective action by *Geloina eros*.

Acknowledgement

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Table 1
Composition of the experimental diet in each group for test CCl_4 and *Geloina eros* extract

Ingredient (%)	Diets						
	Control	*Geloina eros* extract	CCl_4	CCl_4 + *Geloina eros* extract	CCl_4 + *Geloina eros* extract	CCl_4 + *Geloina eros* extract	CCl_4 + Silymarin
	(A)	(B)	(C)	(D)	(E)	(F)	(G)
Sucrose	20	20	20	20	20	20	20
Casein	35	35	35	35	35	35	35
Corn starch	30	24	30	24	23	22	18
Cellulose	5	5	5	5	5	5	5
Corn oil	5	5	5	5	5	5	5
Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline	0.2	0.2	0.2	0.2	0.2	0.2	0.2
AIN Mineral mix[(a)]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
AIN Vitamin mix[(b)]	1	1	1	1	1	1	1
Geloina eros extract	0	6	0	6	7	8	0
Silymarin	0	0	0	0	0	0	12

[(a)] Minerals per 100 g diet: NaCl 7.4 g, $K_2C_6H_5O_7$ H_2O 22g, K_2SO_4 5.2 g, $CaHPO_4$ 50 g, MgO 2.4 g, $FeC_6H_5O_7$ $5H_2O$ 0.6 g, $MnCO_3$ 0.35 g, $CuCO_3$ 30 mg, $CrK(SO_4)_2$ $12H_2O$ 55mg, $CoCl_2$ $6H_2O$ 10 mg, KI 1 mg, $ZnCO_3$ 160 mg.

[(b)] Vitamin per 100 g diet: thiamine 100 mg, riboflavin 150 mg, pyridoxine HCl 100mg, nicotinamide 1000 mg, D-panthenate 500 mg, folic acid 50 mg, vitamine B_{12} 0.1 mg, vitamin A 2.5×10^5 IU, vitamin E 100 mg, calciferol 2×10^4 IU, vitamin C 3.7×10^3 mg.

Table 2
Effect of CCl_4 and *Geloina eros* extract on the body weight and the ratios of liver and kidney weight to body weight of rats[1]

Treatment	Control	*Geloina eros* extract	CCl_4	CCl_4 + *Geloina eros* extract	CCl_4 + *Geloina eros* extract	CCl_4 + *Geloina eros* extract	CCl_4 + Silymarin
	(A)	(B)	(C)	(D)	(E)	(F)	(G)
Body weight (*g*)	283 ± 16^{b}	289 ± 15^{b}	251 ± 12^{a}	285 ± 16^{b}	287 ± 13^{b}	288 ± 16^{b}	285 ± 16^{b}
Liver weights to body weight (%)	2.12 ± 0.11^{b}	2.13 ± 0.13^{b}	1.66 ± 0.12^{a}	2.16 ± 0.13^{b}	2.15 ± 0.15^{b}	2.21 ± 0.12^{b}	2.16 ± 0.13^{b}
Kidney weights to body weight (%)	2.33 ± 0.12^{b}	2.29 ± 0.11^{b}	2.01 ± 0.13^{a}	2.32 ± 0.13^{b}	2.33 ± 0.12^{b}	2.31 ± 0.13^{b}	2.32 ± 0.12^{b}

[1] Data represent mean ± S.D. (n=8). Values in the same row are not significantly different, and those in the same column with different superscripts (a-b) are significantly different ($p < 0.05$).

Table 3
Effects of *Geloina eros* extract on hepatic histopathology of liver damage in rats treated with CCl_4

Parameter	Design of treatment						
	Control	*Geloina eros* extract	CCl_4	CCl_4 + *Geloina eros* extract	CCl_4 + *Geloina eros* extract	CCl_4 + *Geloina eros* extract	CCl_4 + Silymarin
	(A)	(B)	(C)	(D)	(E)	(F)	(G)
Hepatocellular degeneration	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 0.6*	1.6 ± 0.3[a]	1.5 ± 0.2[a]	1.2 ± 0.2[a]	1.6 ± 0.2[a]
Central lobular necrosis	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 0.5*	1.3 ± 0.2[a]	1.3 ± 0.3[a]	1.3 ± 0.1[a]	1.5 ± 0.2[a]
Hepatic lipidosis	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 0.3*	1.2 ± 0.3[a]	1.2 ± 0.2[a]	1.5 ± 0.3[a]	1.3 ± 0.1[a]
Hepatic fibrosis	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 0.5*	1.5 ± 0.2[a]	1.3 ± 0.3[a]	1.3 ± 0.2[a]	1.2 ± 0.3[a]
Cholangiocyte hyperplasia	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 0.3*	1.3 ± 0.3[a]	1.5 ± 0.1[a]	1.2 ± 0.2[a]	1.5 ± 0.2[a]

Values are expressed as mean ± SD (n = 8) in each group.

0, no histopathologic change; 1≦, mild histopathologic change; 2≦, moderate histopathologic change; 3≦, severe histopathologic change.

[a] Significant difference at $P<0.05$ levels compared with the CCl_4.

* $P<0.05$ indicate statistically significantly different from control.

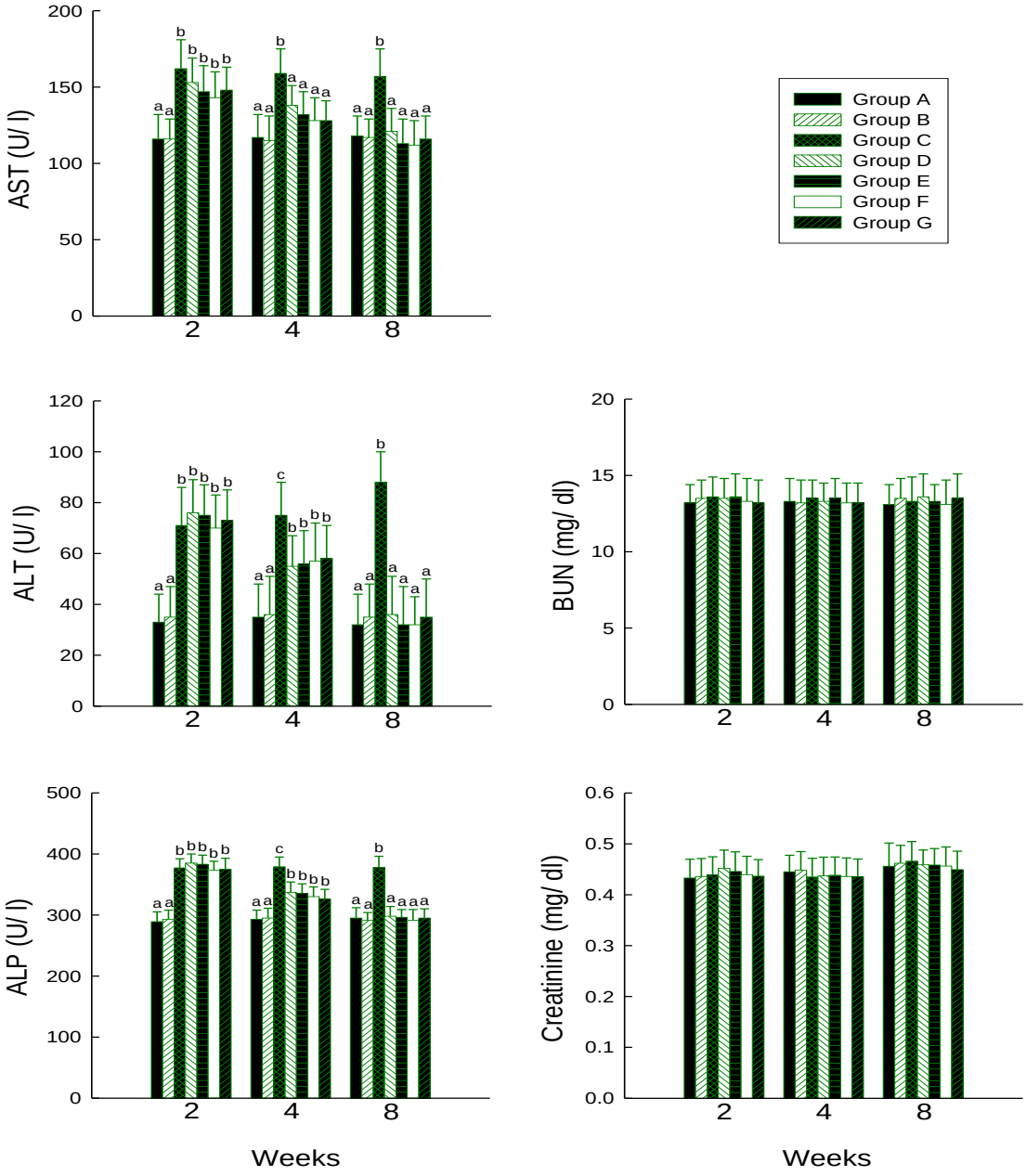

Fig. 1. Effect of CCl_4 and *Geloina eros* extract in levels of BUN, creatinine, AST, ALT and ALP of rats. a-c: values in the same week with different superscript are significantly different (P<0.05). Group A= received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B=6% of *Geloina eros* extract, Group C=CCl_4, Group D= CCl_4 (6% of *Geloina eros* extract), Group E= CCl_4 (7% of *Geloina eros* extract) , Group F= CCl_4 (8% of *Geloina eros* extract) , Group G= CCl_4 (12% of silymarin).

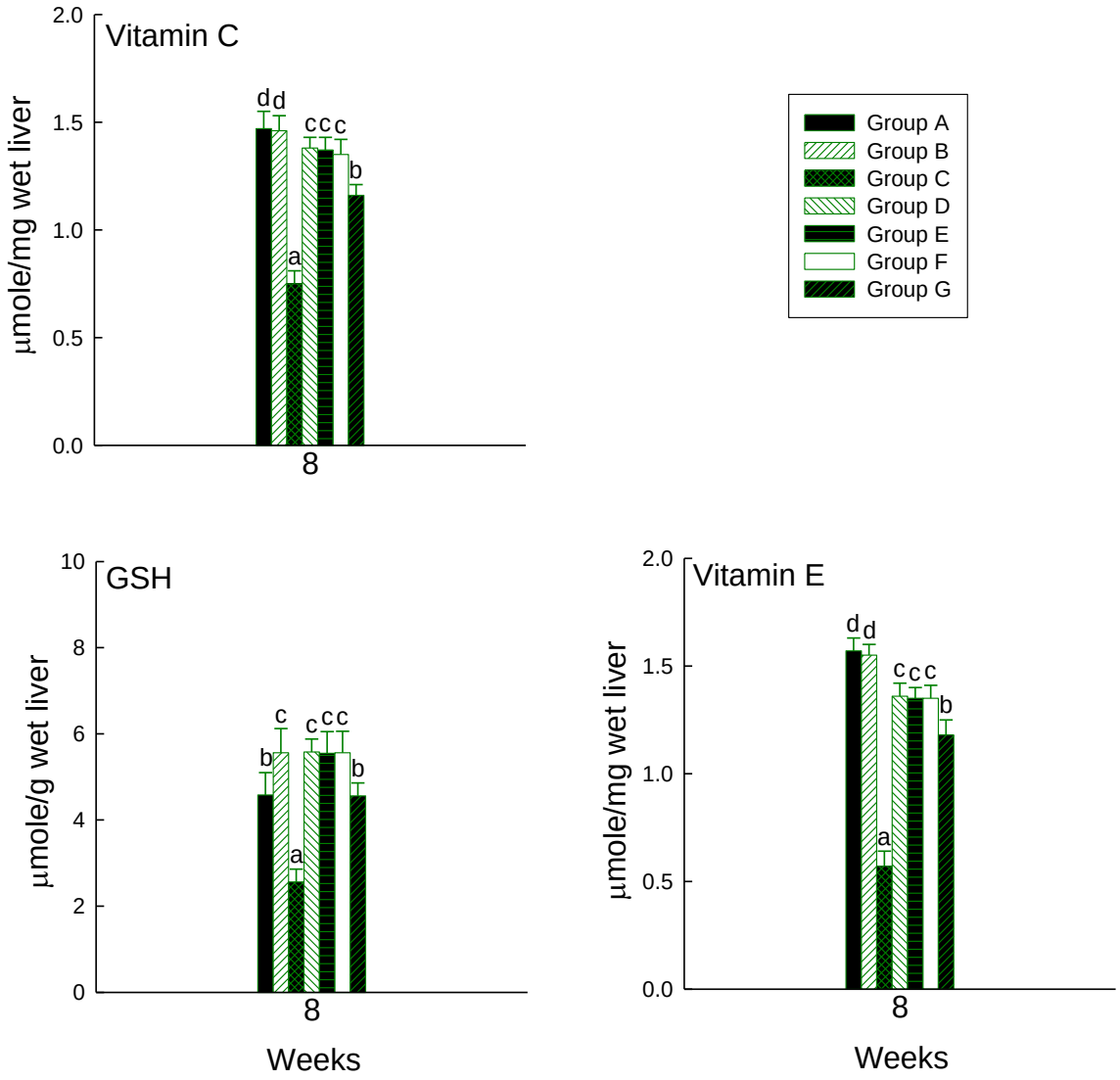

Fig. 2. Effect of CCl_4 and *Geloina eros* extract in levels of Vitamin C, E, and GSH in the liver of rats. a-d: values in the same week with different superscript are significantly different ($P<0.05$). Group A= received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B=6% of *Geloina eros* extract, Group C=CCl_4, Group D= CCl_4 (6% of *Geloina eros* extract), Group E= CCl_4 (7% of *Geloina eros* extract) , Group F= CCl_4 (8% of *Geloina eros* extract) , Group G= CCl_4 (12% of silymarin).

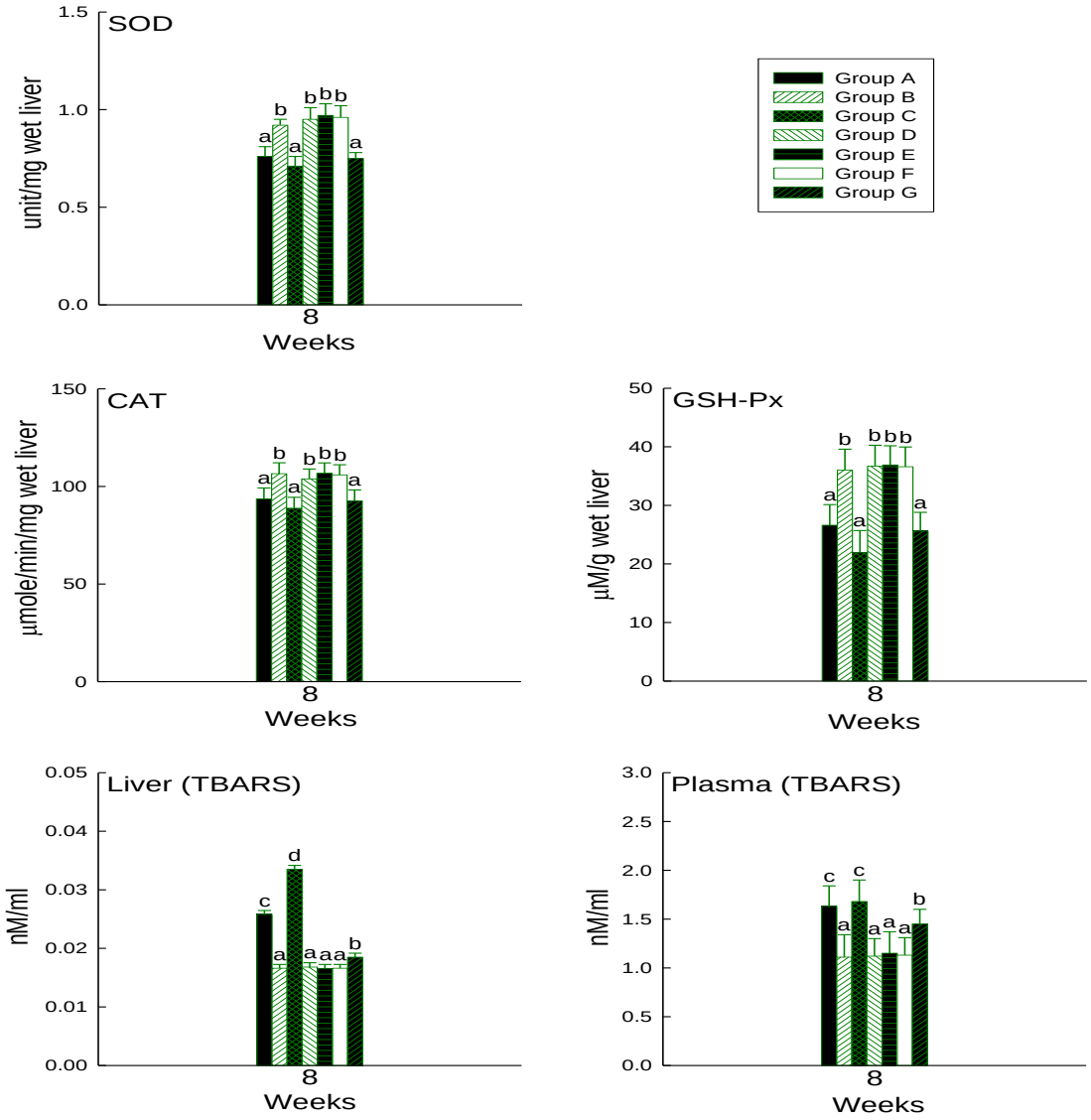

Fig. 3. Effect of CCl_4 and *Geloina eros* extract on the activity of SOD, CAT and GSH-Px in the liver and plasma and hepatic TBARS concentrations of rats. a-d: values in the same week with different superscript are significantly different (P<0.05). Group A= received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B=6% of *Geloina eros* extract, Group C=CCl_4, Group D= CCl_4 (6% of *Geloina eros* extract), Group E= CCl_4 (7% of *Geloina eros* extract) , Group F= CCl_4 (8% of *Geloina eros* extract) , Group G= CCl_4 (12% of silymarin).

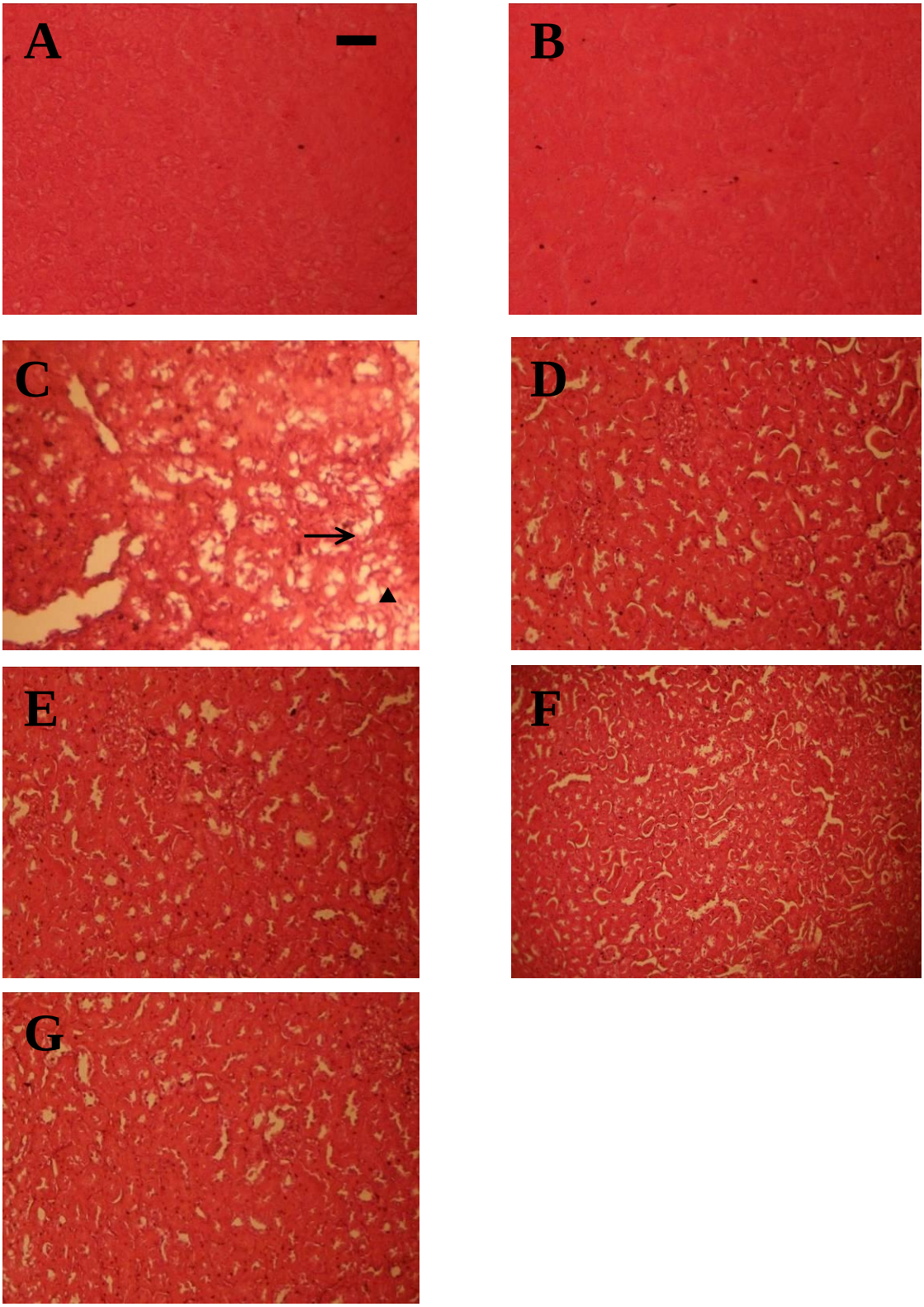

Fig. 4. Microscopic cross section of liver lobules in rat after 8 weeks (x 400 H&E). Bar represents 0.01 mm. Group A= received the basal or control diet. All other groups were fed the basal diet with the addition of the following: Group B=6% of *Geloina eros* extract, Group C=CCl_4, Group D= CCl_4 (6% of *Geloina eros* extract), Group E= CCl_4 (7% of *Geloina eros* extract) , Group F= CCl_4 (8% of *Geloina eros* extract) , Group G= CCl_4 (12% of silymarin). (→, hepatocellular necrosis;▲, hepatocyte fibrosis).

一、前言

台灣常見貝類是台灣重要的漁獲資源，在利用時，多經過去儲外殼等外部特徵，在經過一連串後續加工程序或貯藏，如:冷藏、冷凍與加熱等步驟，由於可供辨識的外部特徵消失，因此需利用其他方法來鑑定貝類物種。而蛋白質分析方法較難應用在加工品貝類物種鑑定上，故本實驗選用分析基因序列來鑑定貝類物種，而儲藏與加工時間及程度的差異皆會影響基因序列的完整性，進而影響到基因鑑定貝類物種的成功與否，故鑑定加工之物種時需先觀察其DNA裂解情形，選用合適的長度的目標基因進行引子設計，再搭配相關的基因方法，以辨別貝類物種。

因此，本部分實驗將探討加工與儲藏條件對貝類基因序列完整性之破壞程度，觀察其DNA裂解程度，並建立針對台灣十一種常見貝類的基因序列資料與基因技術設計合適的引子及方法建立貝類加工與儲藏品之鑑定方法。

二、摘要

本研究針對十一種市售常見貝類，包括馬蹄蛤(Geloina erosa)、黃金蜆(Corbicula fluminea)、文蛤(Meretrix lusoria)、環文蛤(Cyclina sinensis)、蝦夷扇貝(Mizuhopecten yessoensis)、毛蟶(Sinonovacula constricta)、菲律賓簾蛤(Ruditapes philippinarum)、綠殼菜蛤(Perna viridis)、海瓜子(Ruditapes philippinarum)、蚵仔(Crassostrea gigas)、花蛤(Gomphina aequilatera)等，進行粒線體基因組COI(cytochrome oxidase I)PCR-RFLP鑑定及分析。

貝類樣品經收集採肉後，進行DNA萃取，再利用自行設計之共通性引子COI-F 和 COI-R進行粒線體基因組之部分COI基因片段擴增，所得之片段長度為460 bp，PCR產物經純化與定序後，利用PCR-RFLP限制酶切位鑑定此十一種市售常見貝類。研究結果顯示，限制酶切位分析可將此十一種市售常見貝類進行區別。

市售貝類常見加工產品容易發生內容不實或是摻雜其他物種，因此所建立之基因資料於未來研究上，可作為分子鑑定檢測技術基因引子與檢測探針設計之參考依據，所開發之技術亦可供相關衛生單位作為貝類及其加工品快速檢測之用。

研究背景

本實驗樣品台灣常見貝類是台灣重要的漁獲之一，物種有馬蹄蛤、文蛤、黃金蜆、綠殼菜蛤、扇貝、毛蟶、海瓜子、蚵仔、花蛤、赤嘴蛤是國人飲食中常見的貝類物種。

基因序列COI 位於粒線體基因組，屬於變異性較大的序列，也是現今物種基因資料庫上的主流序列，COI 在魚類、昆蟲及兩棲爬行動物等物種鑑定上有相當高的可信度。

由於蛋白質易受貯存與加工過程的影響而造成流失或變異，導致實驗進行上之困難，隨著基因技術的進步，分類學家將鑑定物種的目標逐漸轉移至穩定不易變異的DNA上，而所使用的基因技術基礎多建立在以聚合酶反應(polymerse chain reaction,PCR)方法增幅基因片段上，常用的基因分析方法有直接定序，隨機放大多型性DNA(random amplified polymorphic DNA)、單股結構多樣性(single strand conformation polymorphism；SSCP)以及限制酶切割片段長度多型性(restriction fragment length polymorphism；RFLP)等，目前的基因鑑種分析方法其目標多以粒線體(mitochondria, mt)所含之DNA為主。

三、材料與方法

(一)、實驗材料

取馬蹄蛤(Geloina erosa)、黃金蜆(Corbicula fluminea)、文蛤(Meretrix lusoria)、環文蛤(Cyclina sinensis)、蝦夷扇貝(Mizuhopecten yessoensis)、毛蚵(Sinonovacula constricta)、菲律賓簾蛤(Ruditapes philippinarum)、綠殼菜蛤(Perna viridis)、海瓜子(Ruditapes philippinarum)、蚵仔(Crassostrea gigas)、花蛤(Gomphina aequilatera)之新鮮貝類唇部肌肉以45℃ 乾製4小時以及100℃烹煮30分鐘和121℃高壓滅菌15分鐘後進行DNA萃取，另取新鮮貝肉於-20 ℃凍藏1個月後再進行DNA粗萃。

(二)、實驗方法

1. DNA之萃取

本方法依照Desalle and Birstein(1996)、Lopez等(1994)以及Sambrook等(1991)之方法稍加修改而成，其步驟簡述如下：

取0.1 g之樣品置入吳俊之1.5 ml微量離心管中，加入0.5 ml萃取液(50 mM Tris-HCl，pH=8.0、1%SDS、0.2 M NaCl、0.1 M EDTA，pH=8.0、5 mg/ml proteinase K)(Amresco,Solon,OH)，以55℃水浴並輕微搖動4小時以上或隔夜，以分解樣品。

接著利用酚(phenol)和氯仿 (chloroform) 兩種有機溶劑來除去蛋白質及多醣類，phenol可以除去蛋白質，而chloroform可以除去殘餘液中phenol。其標準步驟如下：加入同體積的酚飽和溶液(Amresco,Solon, OH)(1：1, v/v)，輕搖使其均勻混和後，以12000 x g，4℃離心10分鐘，吸取上層液至乾淨之1.5 ml微量離心管，重複加入 phenol 在萃取一次，再加入等體積之chloroform(Amresco,Solon, OH)，同樣經過離心後取上層液，加入二倍體積之無水酒精，輕搖使其均勻混和後，置於 -20℃下3小時使DNA沉澱。取出後以12000 x g，4℃離心20分鐘後，取沉澱物，再加入200 µl之70%酒精清洗後，以12000 x g ，4℃離心10分鐘，去上清液後再抽乾沉澱物中的酒精和水份。待其乾燥後加入50-100µl之二次無菌蒸餾水，溶解沉澱物，此為粗萃DNA，儲存於 -20℃備用。

2. 基因引子(primer)之設計

本實驗所使用之基因引子則是針對十一種台灣常見貝類所設計之特異性引子，所增幅出基因片段大小為460 bp，其位置坐落於COI gene上。

COI-F: 5’-GAT ATA DCT TTT CCG CGT NTA AAT - 3’

COI-R: 5’-ACT TCA GGA TGH CCA AAA AAY CAA - 3’

3. PCR增幅反應

PCR增幅反應總體積100 µl，其中包括粗萃之DNA(100-1000 ng)、primer0.4 µM、dNTP 200 µM、5U的Pro Taq DNA polymerse(Promega, Wisconsin, USA)及 PCR buffer (20 mM Tris-HCl pH=8.0、15 mM MgCl2、1%Triton X-100、0.1 mM EDTA、1 mM DTT、50% glycerol)。利用溫度循環控制儀(Techne Ltd.)進行PCR增幅反應，其反應條件如下：

Step 1 ：95℃ 10分鐘，使DNA雙股變性打開。

Step 2 ：30次循環反應：

(1). 95℃ 1分鐘(denatrue)。

(2). 49℃ 1分鐘(annealing)。

(3). 72℃ 1分鐘(extension)。

Step 3 ：72℃ 10分鐘作為最後延伸反應。

4. 電泳分析

粗萃之DNA及PCR產物分別取5 µl用0.8%以及1.2%之agarose gel做電泳分析，電泳完成後，以10 mg/ml溴化乙錠EtBr(ethidium bromide)溶液染色1分鐘，取出後以清水退染10-20分鐘，再以UV照相系統(Hitachi Image VDS System,Hitachi Co.,Tokyo)照相並存檔，以確定DNA片段大小和濃度。

5. PCR產物之純化與定序

取PCR產物載入2.0%之agarose gel以50伏特電壓透析電泳。電泳後將膠片置入濃度約為10 mg/ml之EtBr溶液中1分鐘，取出後以清水退染10-20分鐘，置於UV燈下並將PCR產物位置之亮帶膠塊切下，加入5倍體積之Tris-EDTA(TE)buffer，置於65℃經5分鐘待其溶解後，以1倍體積之phenol，輕微翻轉混勻後以12000 x g、4℃離心10分鐘，取上層液加入0.5 ml phenol-chloroform混勻後如上述方式離心並抽取

上層液再加入0.5 ml之chloroform，同樣在經過離心後取上層液，以0.1倍體積之3M醋酸鈉NaOAC(sodium acetate，pH =5.2)與兩倍體積之100%乙醇溶液進行沉澱反應(-20℃，3小時)，取出後再經過12000 x g、4℃離心20分鐘後，所得之沉澱物再以70%之乙醇溶液清洗並離心，取沉澱物溶於20μl之二次無菌蒸餾水中，2μl跑電泳進行確認。

PCR產物送至明欣生物科技有限公司(台北，台灣)進行定序，使用sequencer為：ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit(PE Applied Biosystem,CA)；定序後所得兩段互補序列經由國家衛生研究院提供之巨分析服務系統(Genetics Computer Group,2000)分析比對，並尋找合適之限制酶及其切位以做為各貝類物種之辨別。

限制酶分析PCR產物

分別取純化後所得之PCR產物5 μl，置入1.5 ml微量離心管中，並加入各1 μl之限制酶、10 x buffer、10 x 牛血清(bovine serum albumin,BSA)及2 μl之二次無菌蒸餾水，使總體積為10 μl。分別以限制酶之最適溫度水浴1-2小時，取出後混合1 μl loading dye，並注入2.0%agarose gel，再以100伏特電壓進行電泳分析，取出此膠片置入濃度約為10 mg/ml EtBr溶液中1分鐘，取出後以清水退染10-20分鐘，再以UV照相系統照相並存檔。

五、結果

經新鮮、冷凍、乾製、100℃、加熱處理過之貝類唇肉粗萃DNA，結果顯示新鮮貝肉中所萃取之DNA幾乎沒有裂解的情況其大部分長度仍保持在15000 bp以上，於-20℃以下貯藏三個月之魚肉粗萃顯示有部分裂解情形，長度大約在100 – 10000 bp 之間；而已55℃烘乾4小時其DNA裂解嚴重 ，長度約在100-5000 bp之間；而以烹煮加熱100℃ 30分鐘，其DNA裂解更為嚴重，長度在100-1000 bp之間；高壓滅菌121℃ 15分鐘，其DNA裂解最為嚴重，長度100-500 bp之間。將粗萃DNA以COI-F及COI-R兩管PCR引子進行增幅反應，於高壓滅菌之貝類機肉粗萃DNA無法成功增幅。由結果顯示，貝類唇肉經新鮮、烹煮、乾製均可成功增幅出460 bp長度之基因片段 (Fig. 1)，經過純化及定序之後，由序列資料顯示加工程度並不會影響貝類之基因序列。

根據前述所建立的方法來對不同加工程度之貝類唇肉DNA做限制酶切位分析，首先使用限制酶BsaJI針對新鮮、冷凍、乾製、100℃烹煮30分鐘等不同加工程度之貝類肌肉基因PCR產物進行切位分析試驗，不論是何種加工程度之貝類肌肉，其PCR產物皆可以BSAJI將十一種貝類區分為兩個不同組別，合併使用BmrI之限制酶切位分析結果，可鑑別出不同加工程度之四種貝類種別。在進行限制酶BsaJI之切位分析時，在馬蹄蛤的部分可被切為247+213 bp的長度(Fig. 2)，使用BmrI限制酶切位時，在環文蛤的部分可被切為102+358 bp，在牡蠣的部分可被切為147+313，在海瓜子的部分可被切為202+258 bp(Fig. 3)；另外選用MslI限制酶針對前兩管限制酶未區分的貝類進行切位可將未分類六種貝類再區分為兩個組別，利用MslI限制酶切位分析，在蝦蜆扇貝的部分可被切為300+160 bp，在菲律賓簾蛤的部分可被切為184+276 bp，在花蛤的部分可被切為185+275 bp，菲律賓簾蛤和花蛤因切位相似無法明顯於MslI限制酶切位分析後電泳判讀(Fig. 4)，因此，選用NlaIII限制酶切將毛蟹、文蛤、菲律賓簾蛤進行限制酶切位分析，在毛蟹的部分可被切為145+315，在文蛤的部分可被切為184+276，菲律賓簾蛤的部分則被切為185+275(Fig. 5)，因此在限制酶切位後電泳分析可清楚判讀，此外，將菲律賓簾蛤和花蛤利用限制酶NlaIII進行切位分析，菲律賓簾蛤可被切為185+275，花蛤則無法被切割，經過電泳分析後，可明顯判讀(Fig. 6)；再選用HaeIII限制酶切位分析將綠殼菜蛤、黃金蜆進行切位分析，在黃金蜆的部分可被切為295+102+63，在綠殼菜蛤的部分則無法被切位(Fig. 7)；結果顯示，利用限制酶BsaJI、BmrI、MslI、NlaIII、HaeIII切位分析可將此十一種台灣常見貝類區別。

六、討論

貝類在台灣是高經濟價值的魚獲，常被當作原料製成多種貝類產品，而市售的貝類製品相對於其他物種製品通常屬於高價位之產品，因此在利差的誘因之下，部分的業者以低價貝類物種製成仿製品高價賣出，造成品牌之不正確性，而在國內外的研究報告中，尚無這類鑑定仿貝類製品之報告，因此本實驗針對這部分去建立相關鑑定技術，以供做檢測仿貝類製品之用，以確保貝類製品的品質，並作為相關單位對貝類製品品牌標是管控之用。

本研究所使用的目標基因片段為粒線體DNA之部分COI基因片段。對於貝類物種鑑別的DNA分析實驗多是以粒線體DNA保留性較高的區域來當作目標基因進行增幅反應，在進行後續的實驗步驟。粒線體DNA在脊椎動物中具有高度保留性，其演化方式為母系遺傳切不經過重組步驟，因此粒線體DNA經常被用來作為種別鑑定與種別分析之研究。

粒線體DNA的部分基因序列COI ，屬於變異性較大的序列，也是現今物種基因資料庫上的主流序列，COI在魚貝類、昆蟲及兩棲爬行動物等物種鑑定上有相當高的可信度。

在PCR增幅，Espiñeira等學者(2009) 利用SALM-H和SALM-L引子針對鮭魚、鱒魚、鯛魚物種之加工罐頭、煙燻、冷凍產品成功增幅出142 bp，結果顯示其經過加工過之罐頭所萃取出之DNA則斷裂至200 bp以下，因此針對加工流程劇烈的產品，其所使用的目標基因片段需適度調整。Atsushi等學者(2011)針對阿拉斯加進口鱈魚明太子產品進行鑑定，結果顯示利用三組引子分別對於阿拉斯加鱈魚、太平洋鱈以及藍鱈進行增幅，阿拉斯加鱈於設計於粒線體ATP6成功增幅出255 bp，大西洋藍鱈設計於cyt b成功增幅出223 bp ，太平洋鱈設計於粒線體ATP6成功增幅出223 bp。Futoshi等學者(2006)同樣以350 bp之部分5SrDNA gene片段作為鯖魚加工產品鑑別。而針對加工較嚴苛之產品鑑定，其選用目標基因片段長度必須適度調整。

在本實驗中，所設計之基因引子COI-F＆COI-R可適用於乾製、冷凍、水煮，故本實驗所建立之方法可解決貝類加工品品牌標示之掌控問題。

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Table 1. Length of restriction fragments obtained using the BsaJI, BmrI, MslI, NlaIII and HaeIII restriction enzyme on the PCR products of eleven shellfish species.

	Enzyme				
Shellfish species	BsaJI	BmrI	MslI	NlaIII	HaeIII
Geloina erosa	247/213	460	460	460	460
Corbicula fluminea	460	460	460	460	292/102/63
Meretrix lusoria	460	460	460	184/276	460
Crassostrea gigas	460	147/313	460	460	460
Cyclina sinensis	460	102/358	460	460	460
Sinonovacula constricta	460	460	460	145/315	460
Perna viridis	460	460	460	460	460
Ruditapes philippinarum	460	460	184/276	188/275	460
Patinopecten yessoensis	460	460	300/160	460	460
Gomphina aequilatera	460	460	185/275	460	460
Paphia undulate	460	202/258	460	460	460

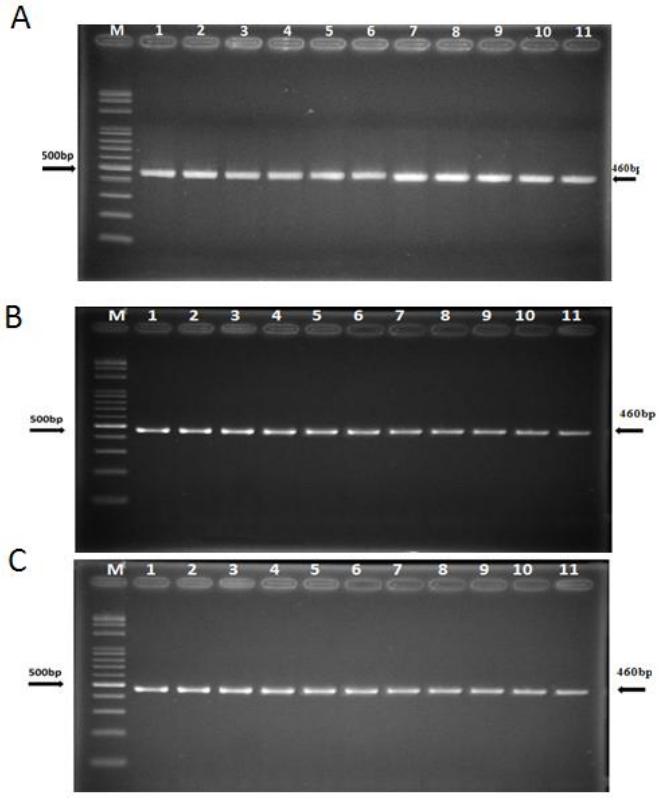

Fig. 1. Electrophoretic analysis of PCR product of the 460 bp COI on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder,A=Fresh sample B=Dried sample C=Boiled sample,1= *Geloina erosa*,2= *Corbicula fluminea*,3= *Meretrix lusoria*,4= *Crassostrea gigas*, 5= *Cyclina sinensis*,6= *Sinonovacula constricta*,7= *Ruditapes philippinarum*,8= *Perna viridis*,9= *Patinopecten yessoensis*,10= *Gomphina aequilatera*,11= *Paphia undulata*.

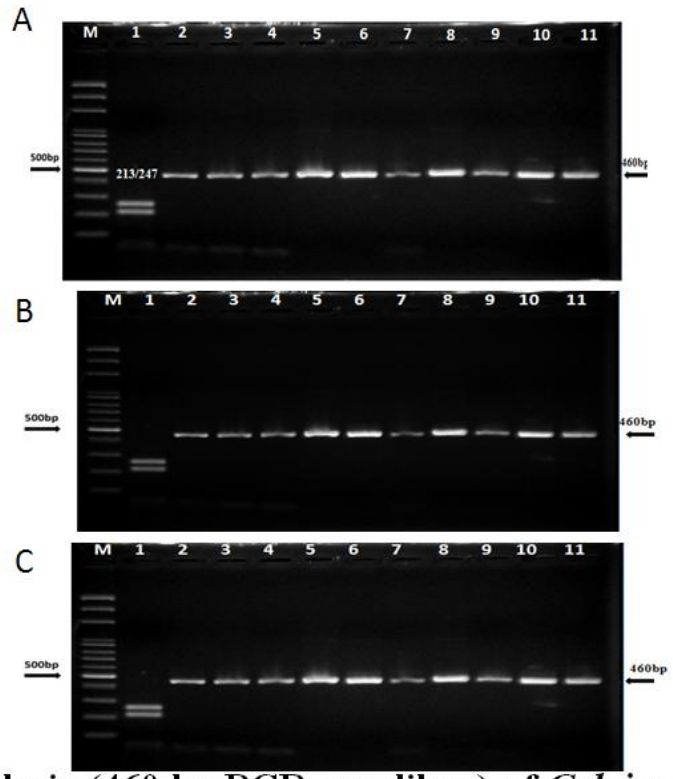

Fig. 2. RFLP analysis (460 bp PCR-amplikon) of *Geloina erosa* with BsaJI on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder, A=Fresh sample B=Dried sample C=Boiled sample,1= *Geloina erosa*,2= *Corbicula fluminea*,3= *Meretrix lusoria*,4= *Crassostrea gigas*, 5= *Cyclina sinensis*,6= *Sinonovacula constricta*,7= *Ruditapes philippinarum*,8= *Perna viridis*,9= *Patinopecten yessoensis*,10= *Gomphina aequilatera*,11=*Paphia undulata*.

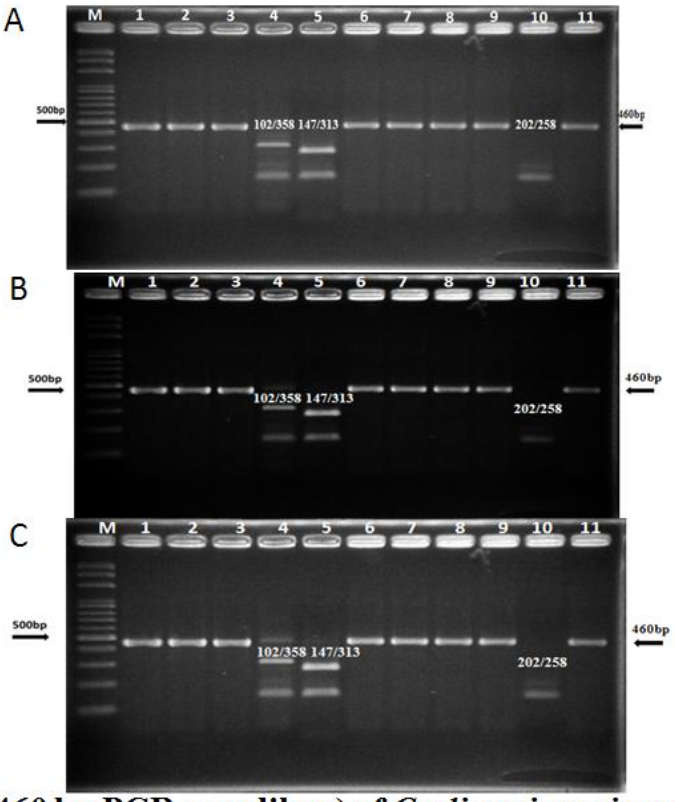

Fig. 3. RFLP analysis (460 bp PCR-amplikon) of *Cyclina sinensis* and *Crassostrea gigas* and *Paphia undulata* with BmrI on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder, A=Fresh sample B=Drierd sample C=Boiled sample,1= *Geloina erosa*,2= *Corbicula fluminea*,3= *Meretrix lusoria*,4= *Crassostrea gigas*, 5= *Cyclina sinensis*,6= *Sinonovacula constricta*,7= *Ruditapes philippinarum*,8= *Perna viridis*,9= *Patinopecten yessoensis*,10= *Paphia undulata*,11= *Gomphina aeauilatera.*

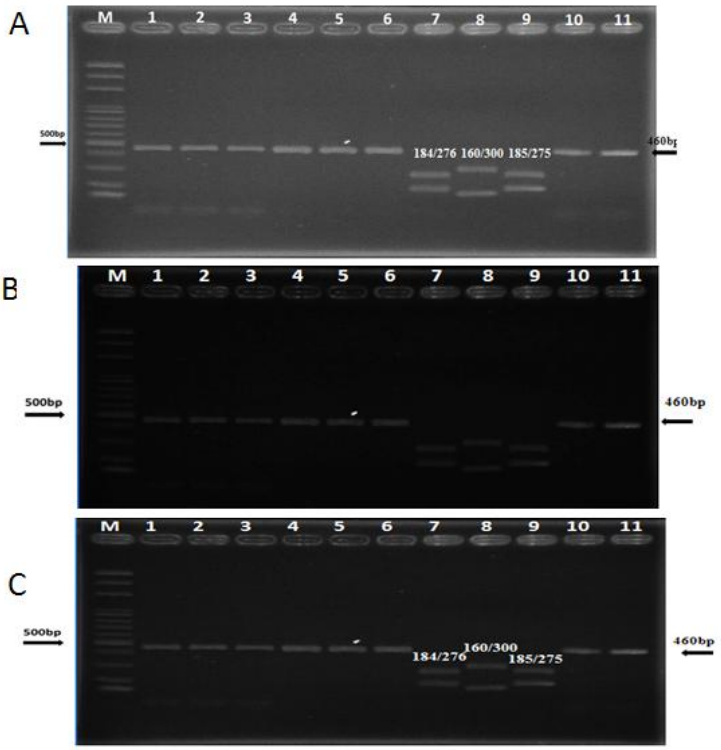

Fig. 4. RFLP analysis (460 bp PCR-amplikon) of Ruditapes philippinarum and Mizuhopecten yessoensis and Gomphina aequilatera with MslI on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder, A=Fresh sample B=Dried sample C=Boiled sample, 1= *Geloina erosa*,2= *Corbicula fluminea*,3= *Meretrix lusoria*,4= *Crassostrea gigas*, 5= *Cyclina sinensis*,6= Sinonovacula constricta,7= Ruditapes philippinarum,8= Perna viridis,9= *Gomphina aequilatera*,10= *Matinopecten yessoensis*,11= *Paphia undulata*.

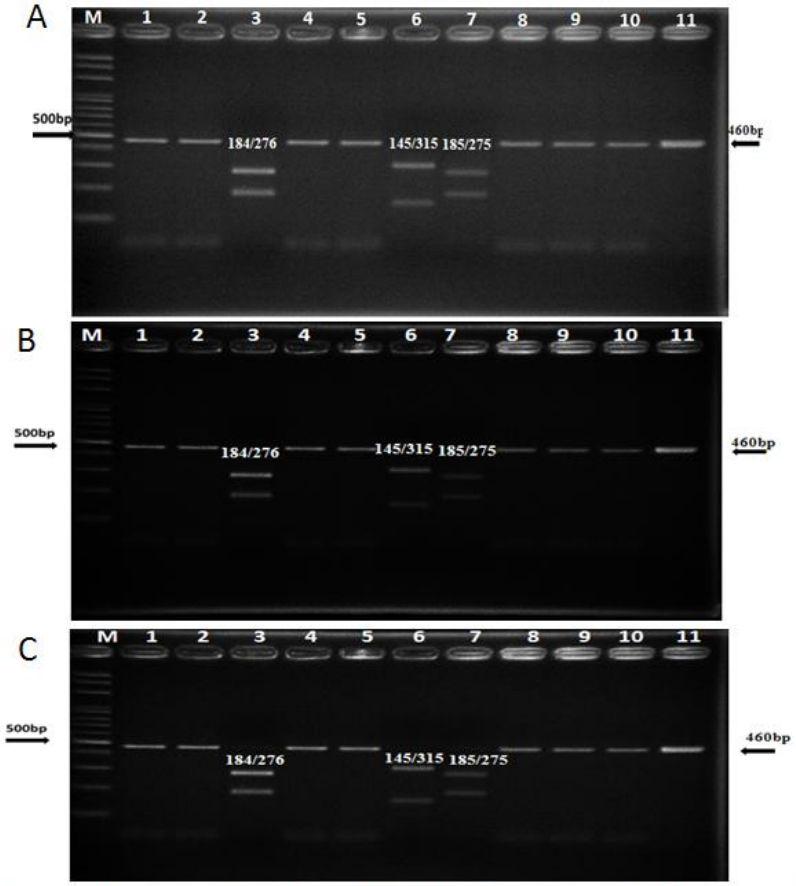

Fig. 5. RFLP analysis (460 bp PCR-amplikon) of Meretrix lusoria and Sinonovacula constricta and Ruditapes philippinarum with NlaIII on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder, A=Fresh sample B=Dried sample C=Boiled sample,1= Geloina erosa,2= Corbicula fluminea,3= Meretrix lusoria,4= Crassostrea gigas, 5= Cyclina sinensis,6= Sinonovacula constricta,7= Ruditapes philippinarum,8= Perna viridis,9= Patinopecten yessoensis,10= Gomphina aequilatera,11= Paphia undulata.

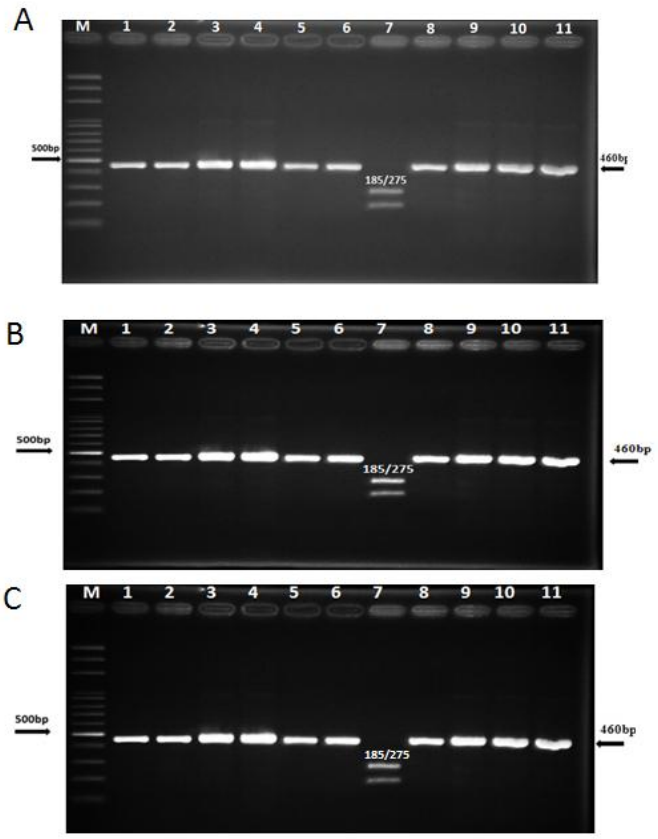

Fig. 6. RFLP analysis (460 bp PCR-amplikon) of *Geloina erosa* with NlaIII on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder, A=Fresh sample B=Dried sample C=Boiled sample,1= *Geloina erosa*,2= *Corbicula fluminea*,3= *Meretrix lusoria*,4= *Crassostrea gigas*, 5= *Cyclina sinensis*,6= *Sinonovacula constricta*,7= *Ruditapes philippinarum*,8= *Perna viridis*,9= *Patinopecten yessoensis*,10= *Gomphina aequilatera*,11= *Paphia undulata.*

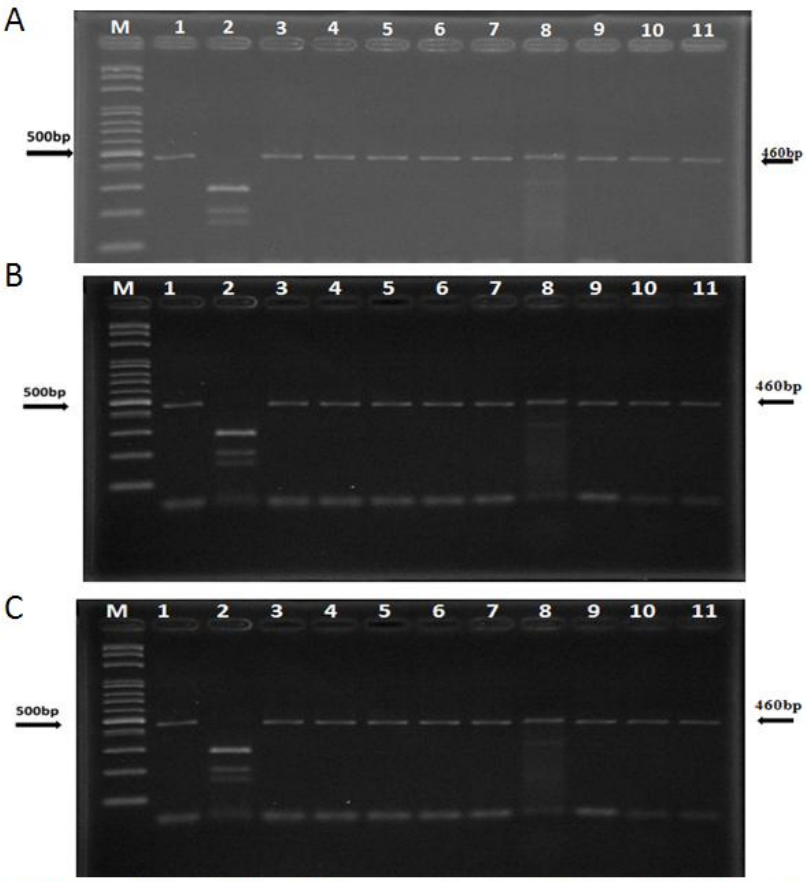

Fig. 7. RFLP analysis (460 bp PCR-amplikon) of *Meretrix lusoria and Sinonovacula constricta and Ruditapes philippinarum* with HaeIII on 2.0% agarose gel. Lane:M=molecular weight marker,Bio 100 bp DNA ladder, A=Fresh sample B=Dried sample C=Boiled sample,1= *Geloina erosa*,2= *Corbicula fluminea*,3= *Meretrix lusoria*,4= *Crassostrea gigas*, 5= *Cyclina sinensis*,6= *Sinonovacula constricta*,7= *Ruditapes philippinarum*,8= *Perna viridis*,9= *Patinopecten yessoensis*,10= *Gomphina aequilatera*,11= *Paphia undulata.*

國科會補助計畫衍生研發成果推廣資料表

日期:2013/10/17

國科會補助計畫	計畫名稱: 探討馬蹄蛤之蛋白質組成、抗氧化活性、保肝作用和PCR-RFLP鑑種技術之建立
	計畫主持人: 葉彥宏
	計畫編號: 99-2313-B-040-006-MY3　　學門領域: 食品及農化

無研發成果推廣資料

99 年度專題研究計畫研究成果彙整表

計畫主持人：葉彥宏	計畫編號：99-2313-B-040-006-MY3
計畫名稱：探討馬蹄蛤之蛋白質組成、抗氧化活性、保肝作用和 PCR-RFLP 鑑種技術之建立	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	1	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 ■達成目標 □未達成目標（請說明，以 100 字為限） □實驗失敗 □因故實驗中斷 □其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：■已發表 □未發表之文稿 □撰寫中 □無 專利：□已獲得 □申請中 ■無 技轉：□已技轉 □洽談中 ■無 其他：(以 100 字為限)
3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)