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Full Length Research Paper

Attenuating effect of *Allium ascalonicum* L. on paracetamol induced seminal quality impairment in mice

Ampa Luangpirom[1]*, Watchara Kourchampa[2] and Thanaree Junaimuang[2]

[1]Applied Taxonomic Research Center, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
[2]Deparment of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

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Overdose and long term use of paracetamol caused testicular toxicity by its toxic oxidative metabolites. Red onion (*Allium ascalonicum* L.) bulbs are rich in quercetin, a strong antioxidant flavonoid which plays a crucial role in cell defense system against oxidative stress. The aim of this study is to determine the attenuating effect of *A. ascalonicum* juice on testicular toxicity induced by paracetamol (0.05 g/100 gBW) in male mice. Blood testosterone and seminal analysis were assessed for testicular toxicity. A significant decrement of blood testosterone and seminal quality impairment were found in groups which received paracetamol. Interestingly, both groups that pre-received *A. ascalonicum* juice at dose 0.5 and 1.0 g/100 gBW for 4 days and concurrent received paracetamol for 14 days revealed a significant increase of blood testosterone and seminal quality improvement in all parameters by increasing total sperm counts, percentage of viable sperms and normal motile sperms, but decreasing percentage of abnormal morphology sperms when compared with the paracetamol treated group. These results indicated that administration of red onion juice can alleviate testicular toxicity induced by paracetamol.

Key words: *Allium ascalonicum* L., paracetamol, seminal quality, testosterone.

INTRODUCTION

Paracetamol is safe at therapeutic dose for analgesic and antipyretic therapy. However, overdose or long term uses of paracetamol have well-konwn adverse effect including hepatotoxicity (Olaleye and Rocha, 2008), depletion of reproductive competence (Ratnasooriya and Jayakody, 2000), alteration of testicular structure and ultrastructure (Yano and Dolder, 2002) and seminal quality impairment (Luangpirom and Maynoi, 2007). These pathogenesis were caused by its oxidant metabolites (N-acetyl-p-benzoquinoneimine,NAPQI) (Mc-Closkey et al., 1999; Yang et al., 2004), which is usually detoxified by conjugation with reduced glutathione in hepatocyte or conjugated with exogenous antioxidant such as vitamin C, vitamin E (Somez et al., 2005), and phytochemicals e.g. quercetin in onion and allicin in garlic (Mahesh and Menon, 2004; Munday and Munday, 2001). This mechanism is a cell scavenging system; the balance of toxic oxidant and antioxidant is a principle mechanism in preventing cell damage and pathogenesis. Shallot or red onion (*Allium ascalonicum* L.) is a versatile vegetable used as ingredient in many Asian dishes and also used as medicinal plants including antimicrobial effect (Dankert et al., 1979), cancer risk reduction (Fukushima et al., 1997), anti-hypertension (Sakai et al., 2003), anti-thrombosis (Ali et al., 1999) and anti-diabetes (El-Demerdash et al., 2005). Its bulbs are a major source of quercetin, a strong antioxidant flavonoid ((Zielinska et al., 2008) which alleviates oxidative stress induced by chemicals or drugs such as oxidative stress in diabetic rats induced by streptozotocin (Mahesh and Menon, 2004). This investigation was to evaluate the protective effect of red onion juice against testicular toxicity from oxidative stress induced by paracetamol. The testicular toxicity was assessed by blood testosterone determination and seminal analysis including total sperm

*Corresponding author. E-mail: amplua@kku.ac.th. Tel: +66-087-4547949. Fax: +66-043-202530.

Table 1. Effect of *A. ascalonicum* juice on blood testosterone level in mice which received paracetamol.

Treatment (g /100 g BW); N=6	Testosterone ($\bar{X}$ + SD, ng /ml)
0	0.32 ± 0.09 [a]
Paracetamol 0.05	0.10 ± 0.03[c]
A. ascalonicum juice 1.0	0.41 ± 0.12[b]
A. ascalonicum juice 0.5 + paracetamol 0.05	0.21 ± 0.02[d]
A. ascalonicum juice 1.0 + paracetamol 0.05	0.20 ± 0.03[d]

N, Number of experimental animals; same alphabet in column indicates non-significant difference (P > 0.05); different alphabet in column indicates significant difference (P < 0.05).

count per animal, percentage of normal motile sperms, abnormal morphology sperms and viable sperms.

MATERIALS AND METHODS

Preparation of *A. ascalonicum* juice

Fresh bulbs of *A. ascalonicum* L. were obtained from grocery in Khon Kaen province, Thailand. They were cleaned, weighed and extracted by juice extractor, then diluted with distilled water at dose of 1.0 and 2.0 g/ml for oral administration (0.5 ml/100 gBW of animal).

Animals

Adult male mice ICR strain (aged 8 weeks old) weighing 35 to 40 g was obtained from the National Laboratory Animal Center of Mahidol University, Salaya, Nakornprathom Province, Thailand. They were housed under a 12:12 of light-day cycle and at 25 ± 1°C, fed with standard pellet diet and were provided with water *ad libitum* at all time. They were acclimatized for one week and the experiments were done after the approval of Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2196).

Experiment

Thirty male mice were distributed into 5 groups of six animals each. Group I received distilled water 0.5 ml/100 gBW for 18 days, Group II received paracetamol, 0.05 g/100 gBW for 14 days, Group III received *A. ascalonicum* juice, 1.0 g/100 gBW for 18 days and Groups IV and V pre-received *A. ascalonicum* juice at dose of 0.5 and 1.0 g/100 gBW, respectively for 4 days and then co-received paracetamol for 14 days. All groups were gavaged daily.

Testosterone determination

At the end of experiment, blood samples of all groups were collected by cardiac puncture under ether anesthesia and serum testosterone was investigated with radioimmunoassay kit (The DSL-400 Active ® Testosterone Coated-tube RIA Kits, Diagnostic System Laboratories, Inc.).

Seminal analysis

After blood sampling, seminal fluid was collected from epididymis and vas deferen. Both side of epididymis and vas deferens of all groups were excised and torn with a syringe needle (No.25) in 2 ml of 0.9% NaCl and incubated at 35°C for seminal determination. Total sperm counts and viable sperms were determined as modified method of Yokoi et al. (2003) by staining seminal fluid with trypan blue and the viable and non-viable sperms with hemacytometer was counted under light microscope. Normal motile sperms were evaluated according to the method of Sonmez et al. (2005) by dropping fresh seminal fluid on slide chamber and covering with cover slip for normal and abnormal motile sperms investigation. The sperm morphology was studied according to the method of Atessahim et al (2006) by staining seminal fluid with Nigrosin-eosin and smear on slide, then quickly dried with air dryer and examined under light microscope. Percentage of normal motile sperms and abnormal morphology sperms were calculated from each total count of 300 sperms per animal.

Statistical analysis

Data of blood testosterone and seminal quality were expressed as mean ± standard deviation ($\bar{X}$ ± SD)). They were assessed by using one way analysis of variance (One way ANOVA). Duncan's multiple test was used to determine the difference among groups (Zar, 1999). Difference were considered to be statistically significant if P < 0.05.

RESULTS

Blood testosterone levels

Testosterone level of the group which received *A. ascalonicum* juice significantly increased than that of the control group. Significant decrease of blood testosterone was found in paracetamol treated group. Both groups which pre-received *A. ascalonicum* juice for 4 days and subsequently co-received paracetamol for 14 days were found significant increment of blood testosterone levels, which significantly differed from the paracetamol treated group (P < 0.05) (Table 1).

Seminal analysis

The group which received paracetamol exhibited an adverse effect on seminal quality by revealing a significant depletion in total sperm count, percentage of

Table 2. Effect of of *A. ascalonicum* (Aa) juice on seminal quality of mice received paracetamol (P).

Treated groups (g/100 gBW); N = 6	Total sperm count $\bar{X}$ ± SD (×10^7 cells/animal	Normal motile sperms ($\bar{X}$ ±SD, %)	Abnormal morphology sperms ($\bar{X}$ ± SD, %)	Viable sperms ($\bar{X}$ ± SD, %)
0	16.27±1.11^a	65.28 ± 3.59^a	32.78 ± 3.20^a	65.04 ± 2.79^a
P 0.05	9.93 ± 1.30^b	17.55 ± 2.47^b	74.22 ± 4.66^b	31.21 ± 3.48^b
A. *ascalonicum* juice, 1.0	17.44 ± 1.18^a	67.93 ± 2.33^a	28.80 ± 4.02^a	67.66 ± 4.41^a
A. *ascalonicum* juice, 0.5 + P 0.05	14.08 ± 1.54^c	56.00 ± 2.17^c	55.60 ± 2.40^c	57.79 ± 2.33^c
A. *ascalonicum,* 1.0+ P 0.05	13.03 ± 3.06^c	53.44 ± 2.92^c	58.67 ± 1.98^c	54.23 ± 4.57^c

N = number of experimental animals; same alphabet in each column indicates non-significant difference (P > 0.05); different alphabet in each column indicates significant difference (P < 0.05)

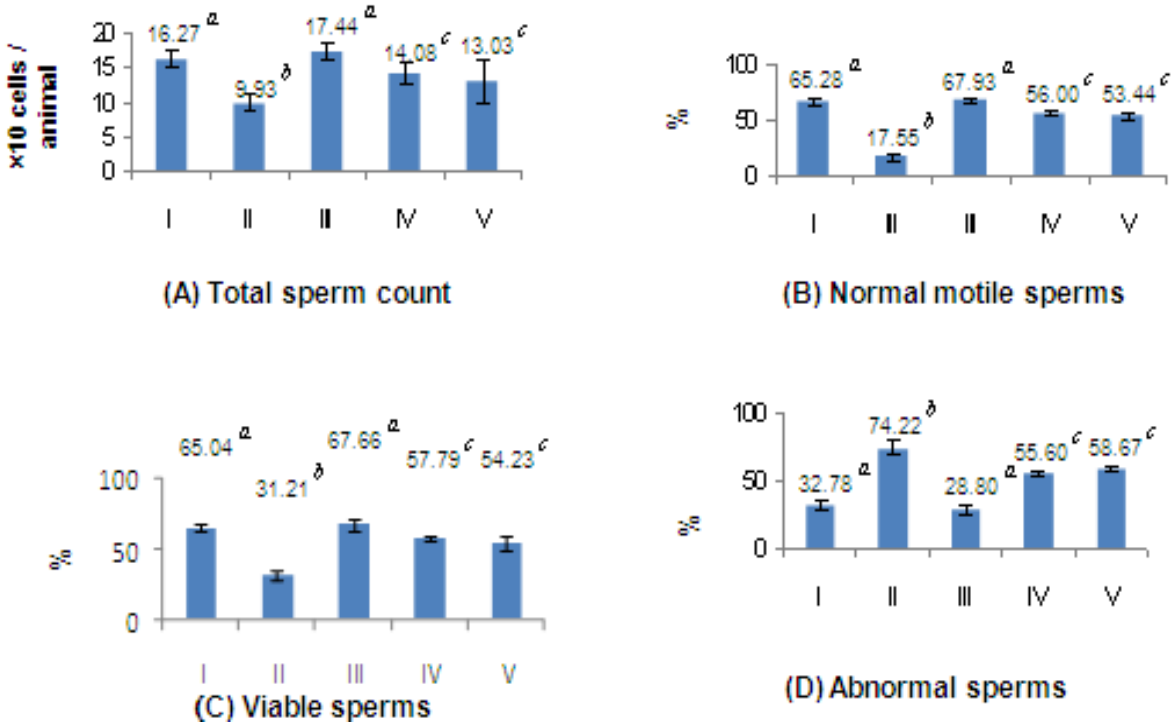

Figure 1. Seminal analysis; I, control; II, paracetamol 0.05 g; III, *A. ascalonicum* juice 1.0 g; IV, *A. ascalonicum* juice 0.5 g + paracetamol 0.05 g; and V, *A. ascalonicum* juice 1.0 g + paracetamol 0.05 g/100 gBW (same alphabet- non significant difference; P>0.05 and different alphabet -significant difference, P < 0.05).

normal motile sperms and viable sperms, but abnormal sperms were found to have significantly increased when compared to the control (P < 0.05). Many forms of abnormal sperms were found including medial protoplasmic droplet, detached head, bent middle piece, bent tail, coiled middle piece and shoehook tail sperms (Figure 2), which were secondary abnormality and occurred during sperm maturation in epididymis (Sorensen, 1979). Incidence of testicular toxicity was not exhibited in the control group and group whichreceived *A. ascolonicum* juice alone. Our study also found protective effect of *A. ascolonicum* juice against testicular toxicity induced by paracetamol in both treated groups, which pre-received *A. ascolonicum* juice for 4 days and subsequently co-received with paracetamol for 14 days by presenting a significant improvement of seminal quality in all parameters (Table 2, Figure 1).

DISSCUSSION

Our results were found that high dose of paracetamol (0.05 g/100 gBW) caused a seminal quality impairment and concurred with a significant depletion of blood

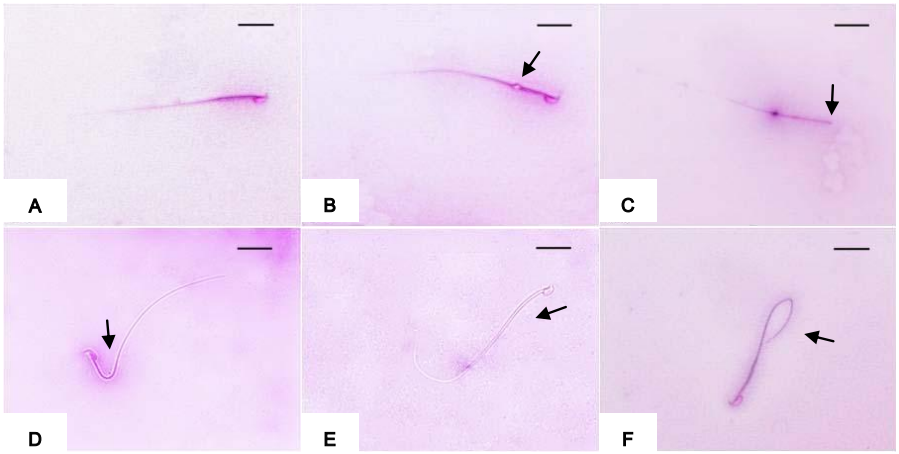

Figure 2. Normal sperm and abnormal sperms of mice (Nigrosin-Eosin stain, bar = 20 µm); A, normal sperm; B-F, abnormal sperms: B, medial protoplasmic sperm; C, detach head sperm; D, bent mid piece sperm; E and F, bent tail sperms.

testosterone level in male mice. Paracetamol (acetaminophen) was suspected to trigger oxidative stress in various organs including testes (Yano and Dolder, 2002), due to the fact that the oxidative damage which occurred in the testes and sperms were highly dependent on their oxidative defense status (Sikka, 2001). Drug metabolizing enzyme activity is also found in the testes, but the oxidative metabolite scavenging system in testes is low as compared to the liver (Seng et al., 1991). Wiger et al. (1995) reported that paracetamol 400 mg/kg BW caused an inhibition of DNA replication that was followed by a reduction of spermatogenesis. Previous studies reported that high dose of paracetamol caused testicular atrophy, oligospermia and decreased male reproductive competence (Boyd, 1970; Ratanassoriya and Jayakody, 2000), triggered apoptosis of spermatocytes and early spermatids and reduction of testicular weight (Yano and Dolder, 2002). From the result of the present study, it was found that both groups which pre-received red onion juice for 4 days and subsequently co-received paracetamol for 14 days revealed the attenuation of testicular toxicity induced by paracetamol, which were assessed by seminal analysis and blood testosterone level. They exhibited an increment of seminal quality in all parameters and showed a significant increase in blood testosterone. According to Zielinska et al. (2008), bulbs of *A. ascalonicum* are a major source of quercetin, which is a well-known flavonoid and a strong antioxidant. In addition, it has been shown to reduce oxidative stress in streptozotocin induced diabetic rats (Khaki et al., 2009; Mahesh and Menon, 2004). Ola-Mudathir et al. (2008) reported that onion juice exerted the protective effect on testicular oxidative damage in rats which received cadmium. Our results affirmed that *A. ascalonicum* juice revealed an attenuation of testicular toxicity induced by paracetamol.

In conclusion, the concurrent administration of red onion (*A. ascalonicum* L.) juice and paracetamol can improve the impairment of testicular function caused by paracetamol; this suggest that onion juice is a useful agent that might protect human health from toxic metabolites induced by paracetamol.

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