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Original Article

Anti-aging Effect of *Panax notoginseng* Saponins via Protecting Endogenous Antioxidant in Fruit Flies

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ABSTRACT

Introduction: *Panax notoginseng* saponins (PNS), the main active ingredients extracted from *Panax notoginseng,* are chemical mixtures, with the five main active components of ginsenosides Rg1, Rb1, Re, Rd and notoginsenoside R1. **Objectives:** The present study investigated the effect of PNS on anti-aging in fruit flies, which represented as a natural physical organism aging model. **Materials and Methods:** After lifelong supplement of PNS in the culture medium at the concentration of 50, 100 and 200 mg/L, the reproduction and the lifespan were observed in fruit flies. The activity of total antioxidative capabilities (T-AOC), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) were detected to evaluate oxidative stress levels. **Results:** Lifelong supplement of 50 mg/L PNS significantly prolonged the lifespan of fruit flies (Log rank χ^2=6.752, *P*=0.009); supplement of 100 mg/L PNS tendency increased the lifespan of fruit flies with no significance; while supplement of 200 mg/L PNS potentially accelerated the death of fruit flies with no significance. Supplement of 50 mg/L PNS protected the reproductive capacity (p<0.05 for 15 and 30 days) in fruit flies. The activity of T-AOC, CAT, total SOD (T-SOD) and GSH-px significantly or tendency increased by 50, 100 and 200 mg/L PNS. **Conclusion:** Supplement of 50 mg/L PNS prolonged the lifespan and protected the reproductive capacity of fruit flies. The mechanism might be, at least in part, through the anti-oxidative stress pathway by protecting the activity of T-AOC, CAT, T-SOD and GSH-px. Supplement of 200 mg/L PNS might be toxic for fruit flies.

Keywords: *Panax notoginseng* saponins, Aging, Lifespan, Reproduction, Antioxidant enzyme.

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INTRODUCTION

Panax notoginseng saponins (PNS) is the main active ingredients extracted from *Panax notoginseng* with various biological activities, including anti-hypertensive,[1] anti-thrombotic, anti-oxidative stress,[2] anti-atherosclerosis, anti-diabetes,[3] neuroprotection,[4] etc. PNS is chemical mixtures, which the five main active components are ginsenosides Rg1, Rb1, Re, Rd and notoginsenoside R1.[5]

The relationship of PNS and aging (including aging related diseases) has been described by literatures. PNS reduced NADPH oxidase 4 expression and decreased reactive oxygen species (ROS) production levels, and further reduced plaque angiogenesis and improved atherosclerosis.[6] PNS enhanced the antioxidant enzymes activity to protect neurons from oxidative stress damage in the brain of Alzheimer's rat.[7] Notoginsenoside R1 improved the learning ability and memory of Alzheimer's rats.[8] These literatures inspire us to wonder whether PNS possesses the effect of anti-aging in natural physical organism aging model.

Therefore, this study was designed to investigate the anti-aging effect of PNS in natural physical organism aging model fruit flies. And the endogenous antioxidant enzymes were determined to interpret the mechanism. This work will provide experimental evidence of PNS for anti-aging application in human aging.

MATERIALS AND METHODS

Materials

PNS (20170325), white-like to pale yellow amorphous powder, was purchased from Yunnan Baiyao Group Wenshan Qihua Co., Ltd., (Yunnan, China). The components of PNS included 32.7% of ginsenoside Rb1, 31.5% of ginsenoside Rg1, 8.5% of notoginsenoside R1, 7.6% of ginsenoside Rd and 4.3% of ginsenoside Re. Glucose (10010518) and antiseptic (1% Ethyl-4-hydroxybenzoate within 75% alcohol) (30086926) were supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Agar (H8145) was supplied by Shanghai Jiafeng Gardening Products Co., Ltd. Yeast (80000193) was supplied by Angel

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Yeast Co., Ltd. (Yichang, China). The activity assay kit of total antioxidative capabilities (T-AOC) (A015), superoxide dismutase (SOD) (A001-2), catalase (CAT) (A007-1) and glutathione peroxidase (GSH-px) (A005) were supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Fruit Fly Strain and the Culture Conditions

The wild type Oregon K line of Drosophila melanogaste fruit flies was used. The fruit flies culture methods and the basal culture medium preparation methods were the same as described in our previous paper.[9] The fruit flies were housed within 50ml plastic vials with 5ml culture medium, and kept in a space with 25±1°C, 60±5% humidity on a 12hr:12hr light/dark cycle. The culture medium was refreshed twice a week. The culture medium was made by 72g glucose, 72g cornmeal, 40ml antiseptic, 10g yeast, 6g agar and water to make 500ml medium. The mixture was cooked and injected into vials.

Lifespan Assay

1520 male fruit flies, eclosion within 8 hr, were randomized into 4 groups: the control group, the 50, 100 and 200 mg/L PNS group. The fruit flies in the PNS groups were housed with culture medium added within corresponding dosage of PNS. Fruit flies in the control group were housed with the basal culture medium. In order to observe the survival time, the amount of dead fruit flies were counted every 3 days (d). The median and mean lifespans were calculated; and the lifespan curve was drawn. The maximum lifespan was obtained by the calculation of the average lifespan of the 10% longest surviving fruit flies.

Reproduction Assay

The fruit flies, eclosion within 8 hr, were randomized into 4 groups: two male and two female fruit flies groups. One group of female fruit flies and one group of male fruit flies belonged to the control group, and the others belonged to the 50 mg/L PNS group. The feeding method was the same as described above. On 15d and 30d, one male and one female fruit flies in the same group were mixed to one vial and consecutively housed with corresponding culture medium. After housing for another 7d, expelled the parent fruit flies. Since the filial generation eclosion of the first fruit fly, the amount of the first filial generation fruit flies eclosion in the following 7d was counted and calculated by sexes, respectively. Ten couple fruit flies parents were observed for each group.

Enzymes Assay

To elucidate the mechanisms of the lifespan prolonging effect of PNS on fruit flies, the effect of PNS on the activity of T-AOC, CAT, SOD and GSH-px was examined. Male fruit flies, eclosion within 8 hr, were randomized into 4 groups: the control group, the 50, 100 and 200 mg/L PNS group. The feeding method was the same as described above. On 0d, 20d and 40d, the fruit flies were collected and weighed. The fruit flies were mixed by body weight (mg) with physiological saline (μl) in a ratio of 1:49 and then homogenized within ice bath. The homogenates were centrifuged twice at 3000 r/min for 15 min and the supernatant was extracted. The activity of T-AOC, CAT, SOD and GSH-px was determined by the manufacturer's instructions.

Statistical Analysis

Data are expressed as mean±SD. Analysis of variance (ANOVA) followed by LSD test was used to compare among groups. The equality of survival curves were evaluated by Log-rank test. Statistically significant difference was considered as $p<0.05$.

RESULTS

PNS Prolonged the Lifespan in Fruit Flies

Compared with the control group, PNS supplement at the concentration of 50 ($p<0.01$) and 100 mg/L prolonged the lifespan of fruit flies, but there is no statistical difference for the 100 mg/L PNS group; while, 200 mg/L PNS potentially accelerated the death of fruit flies with no significance (Figure 1). As shown in Table 1, the mean lifespan parameters further confirmed this result. The mean lifespan of fruit flies was prolonged 4.8% by 50 mg/L PNS supplement (Table 1).

PNS Protected the Reproduction in Fruit Flies

In the fruit fly parents obtained from 15d or 30d, the amount of the total first filial generation ($p<0.05$ for 15d; $p<0.05$ for 30d) significantly increased by 50 mg/L PNS supplement (Figure 2);

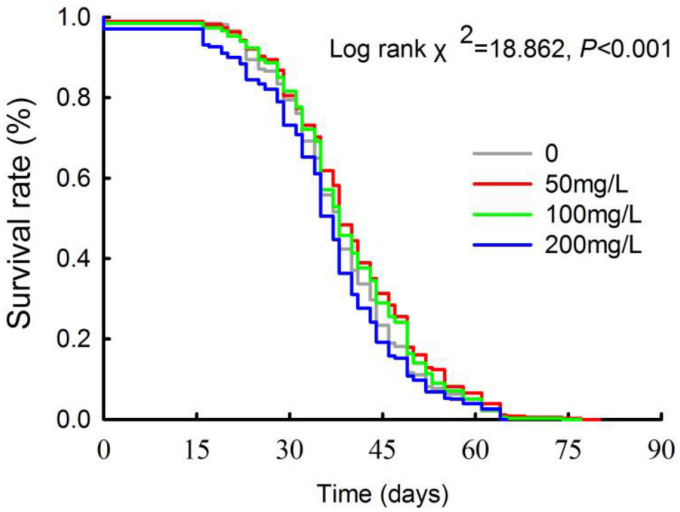

Figure 1: Lifelong supplement of *Panax notoginseng* saponins (PNS) prolonged the lifespan of fruit flies (n=380).

Fruit flies lifelong supplemented with PNS at the concentration of 0, 50, 100 and 200 mg/L. Fruit flies from the control group were housed with the basal culture medium. The numbers of dead fruit flies were counted every 3 days. Log-rank test were used to analysis the Data.

Table 1: Lifespan parameters of lifelong supplement of *Panax notoginseng* saponins (PNS) in fruit flies (*n*=380).

PNS (mg/L)	Mean lifespan (day)	Chang from Control (%)	Log-rank (vs. control)	Maximum lifespan (day)
0	39.6±0.5	-	-	59.1±4.3
50	41.5±0.6	4.8%	$\chi2$=6.752, *P*=0.009	62.5±5.3
100	40.7±0.6	2.8%	$\chi2$=2.321, *P*=0.128	60.6±4.5
200	37.8±0.6	-4.5%	$\chi2$=2.313, *P*=0.128	58.6±4.9

Note: Data are presented as mean±SD.

Maximum lifespan was analyzed by the average lifespan of the 10% longest surviving fruit flies in each group.

PNS=panax notoginseng saponins

Table 2: Reproduction parameters of fruit flies following *Panax notoginseng* saponins (PNS) supplement (*n*=10 couples).

Group	Time	Number of the first final generation			Raising rate vs Control (%)		
		♀	♂	Total	♀	♂	Total
Control	15d	14.9±5.6	15.5±5.9	30.4±10.6	-	-	-
	30d	7.7±5.7	5.9±4.4	13.7±6.6	-	-	-
PNS	15d	18.8±6.3	19.2±6.4	38.0±8.7*	26.2	23.9	25.0
	30d	10.1±4.5	11.1±5.0	21.2±7.5*	31.2	88.1	54.7

Note: Data are presented as mean±SD.

*p<0.05 vs the control group at the same time point.

PNS=panax notoginseng saponins

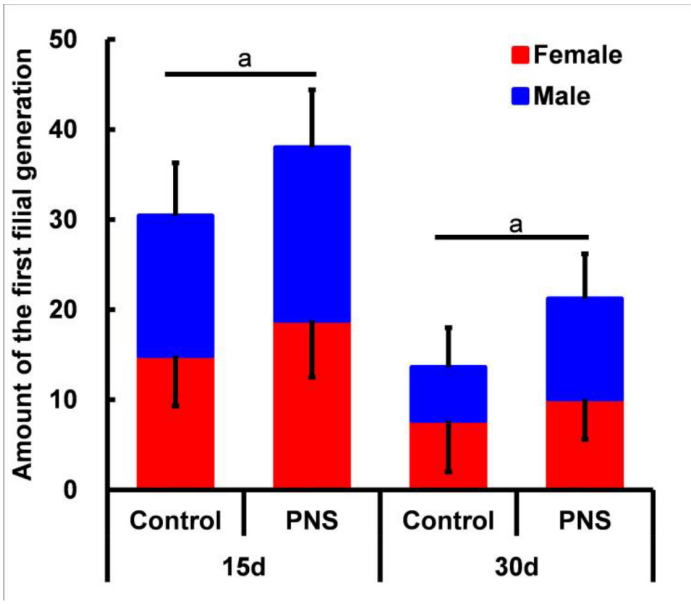

Figure 2: *Panax notoginseng* saponins (PNS) protected the reproduction in fruit flies (*n*=10 couples).

With different sexes separated strictly, fruit flies, eclosion within 8 hr, were grouped and housed. On 15d and 30d, each couple of fruit flies was mixed for 7 days. Since the filial generation eclosion of the first fruit fly, the number of the first filial generation eclosion in the following 7d was observed. Data were presented as mean±SD. and analyzed by ANOVA followed by LSD test. [a]p<0.05 compared with the total amount of fruit flies from the control group at the same time point.

however, there were no significant differences between the amount of the male or female offspring, respectively (Table 2).

PNS Protected the Activity of Antioxidant Enzymes

Compared with the control group, the activity of T-AOC was significantly or tendency protected by 50, 100 and 200mg/L PNS, except the 200 mg/L PNS group on 20d (Figure 3A); the CAT reduction was significantly or tendency inhibited by 50, 100, 200 mg/L PNS (Figure 3B); the total SOD (T-SOD) activity was

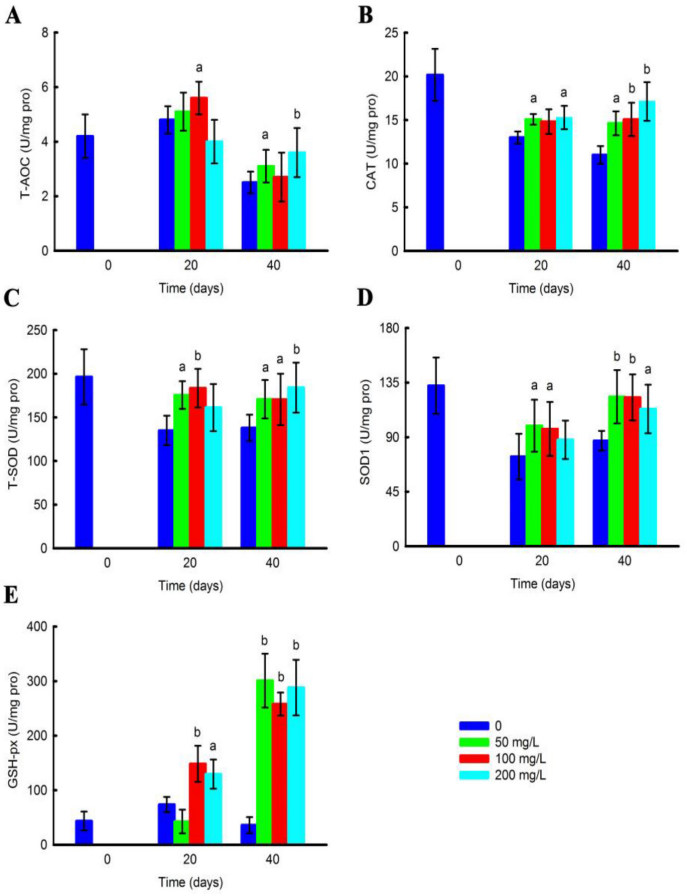

Figure 3: Effect of *Panax notoginseng* saponins (PNS) on antioxidant enzymes in fruit flies (*n*=6).

Fruit flies, eclosion within 8 hr, were supplemented with 50, 100 and 200 mg/L PNS. On 0d, 20d and 40d, fruit flies were collected for the detection of antioxidant enzymes activity. Data were presented as mean±SD and analyzed using ANOVA followed by LSD test. [a]p<0.05, [b]p<0.01 compared with the control group. (A) T-AOC activity. (B) CAT activity. (C) T-SOD activity. (D) SOD1 activity. (E) GSH-px activity. T-AOC, total antioxidative capabilities; CAT, catalase; T-SOD, total superoxide dismutase; SOD1, copper-zinc-containing superoxide dismutase; GSH-px, glutathione peroxidase.

significantly or tendency protected by 50, 100 and 200 mg/L PNS (Figure 3C); the copper-zinc-containing SOD (SOD1) activity was tendency enhanced by 50, 100 and 200 mg/L PNS (Figure 3D); the activity of GSH-px was enhanced by 50, 100 and 200 mg/L PNS, except the 50 mg/L PNS group on 20d (Figure 3E). The protection effect of 50,100 mg/L PNS on antioxidant enzymes was stable; however, the protection of 200 mg/L PNS was unstable.

DISCUSSION

Aging is characterized by structure and function degeneration, adaptability and resistance decline. Aging is divided into two categories, physiological aging and pathological aging. Physiological aging is usually accompanied with an increase of age, which means that lifespan can be used to evaluate the anti-aging effect. Pathological aging is the senile change caused by various factors including many diseases. Most aging models from literature were induced by abnormal factors in healthy animals. These aging models belonged to pathological aging. The advantages of fruit fly using for aging experiments had been described in our previous article.[10-11] So fruit flies were still chosen as physical aging model in the present study. Our results demonstrated that 50 and 100 mg/L PNS supplement prolonged the lifespan of fruit flies. This indicated the anti-aging effect of PNS.

It is consensus that fertility generally decreases with age as a part of senescence.[12] The number of spermatozoa in male human began to decrease at the age of 34, and the probability of pregnancy after sexual intercourse with men over 34 years old decreased with age.[13] Our result showed that 50 mg/L PNS supplement significantly increased the number of fruit fly offspring. This indicated that PNS protected the reproductive capacity in fruit flies. Therefore, we demonstrated the anti-aging effect of PNS in another perspective.

It is well known that aging involves various mechanisms, and one of the most popular studied is oxidative stress. Oxidative stress is defined as imbalance between oxidant system and antioxidant system in cells, thus cause excessive production of ROS.[14] Antioxidant systems in mammals involve many antioxidants such as T-AOC, CAT, SOD, GSH-px, etc.[15-16] They can effectively scavenge ROS and free radicals to prolong the aging process. There are many reports on the relationship of PNS and antioxidants. PNS reduced the accumulation of ROS and protect astrocytes.[17] PNS inhibited the pathway of ROS-TNF-α-p38-VCAM-1 to further inhibit adhesion events to protect arteries.[18] Notoginsenoside R1, one of the five main active ingredients of PNS, inhibited oxidative stress and apoptosis to exert the cardio protective effect on diabetic cardiomyopathy.[19] Notoginsenoside R1 potently inhibited the reduction of CAT, SOD and GSH-px activity induced by ischemia/reperfusion.[20] PNS enhanced the activity of T-AOC, CAT, SOD and GSH-px to inhibit high glucose-induced damage in rat retinal capillary endothelial cell,[21] and protected rabbits from steroid-induced necrosis of the femoral head.[22] Consistently, supplement of 50, 100 and 200 mg/L PNS significantly or tendency increased the activity of T-AOC, CAT, T-SOD and GSH-px in our results. These indicated that the mechanism of the anti-aging effect of PNS involved, at least in part, protecting the antioxidant enzymes.

However, the antioxidant enzymes protecting effect of 200 mg/L PNS was unstable. Associated with the result that 200 mg/L PNS potentially accelerated the death of fruit flies, we inferred that 200 mg/L PNS might be overdose and thus induced toxic effect on fruit flies. This deduction is supported by some other studies. PNS at the concentration of 200 and 400 μg/mL was reported to have cytotoxicity effect on HepaRG cells.[23] Notoginsenoside R1 supplement at the concentration of 50 and 100 μg/mL promoted cell proliferation; while, 200 and 1000 μg/mL supplement inhibited cell proliferation.[24] Further investigations are needed to identify the detailed toxicity mechanism and find the safe dosage range for human application.

CONCLUSION

The present study showed that 50 and 100 mg/L PNS prolonged the lifespan of fruit flies; 50 mg/L PNS protected the reproductive capacity in fruit flies; 50, 100 and 200 mg/L PNS increased the antioxidant enzymes activity, including T-AOC, CAT, T-SOD and GSH-px; while, 200 mg/L PNS might be toxic for fruit flies. This study provides the experimental basis for further study on the anti-aging and anti-oxidative stress effect of PNS. More researches are needed to study the anti-aging effect of PNS and interpret the underlying mechanisms. Meanwhile, the toxic effect induced by overdose of PNS should not be neglected.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PNS: *Panax notoginseng* Saponins; **T-AOC:** Total Antioxidative Capabilities; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **GSH-px:** Glutathione peroxidase; **g:** Gram; **mg:** Milligram; **L:** Liter; **ml:** Milliliter; **μl:** Microliter; **hr:** Hour; **d:** Day; **r:** Round; **min:** Minute.

SUMMARY

The present study investigated the effect of *Panax notoginseng* saponins (PNS), the main active ingredients in *Panax notoginseng*, on anti-aging in fruit flies. After lifelong supplement of PNS in the culture medium, the lifespan and the reproduction of fruit

flies were observed. The activity of total antioxidative capabilities (T-AOC), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) were detected to evaluate oxidative stress damage. Supplement of 50 mg/L PNS prolonged the lifespan (Log rank χ^2=6.752, p=0.009) and protected the reproductive capacity (p<0.05 for 15 and 30 days) of fruit flies. The mechanism might be, at least in part, through anti-oxidative stress pathway involving protecting the activity of T-AOC, CAT, T-SOD and GSH-px. Supplement of 200 mg/L PNS might be toxic for fruit flies.

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