Pharmacogn. Mag.
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ORIGINAL ARTICLE

In Vitro Bioassay-guided Isolation of Radioprotective Fractions from Extracts of *Pinus koraiensis* Bark

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Submitted: 17-09-2016 Revised: 08-11-2016 Published: 18-07-2017

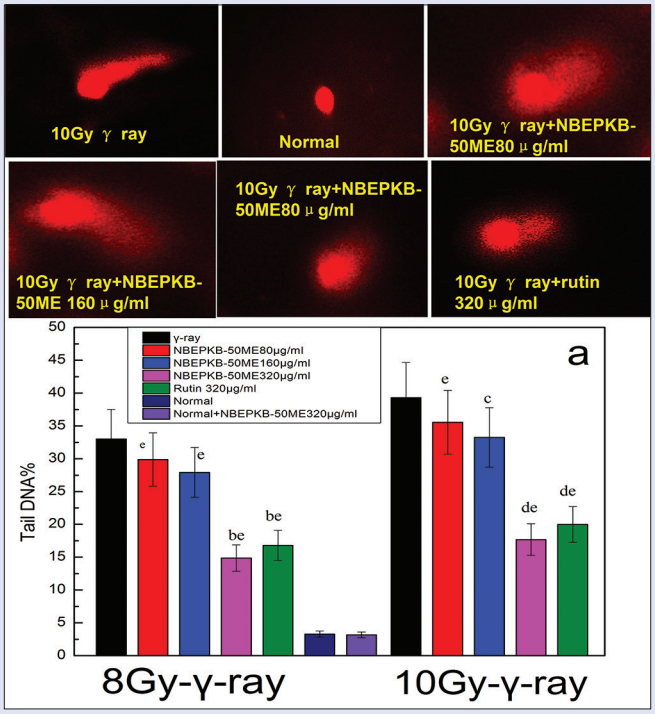
ABSTRACT

Objective: The aim of this study was to evaluate radioprotective effect of extracts of *Pinus koraiensis* bark and its fractions on rat splenocytes by using bioassay-guided isolation in order to obtain the best active fraction. **Materials and Methods:** *P. koraiensis* bark was ground and extracted with water, 40% acetone, 95% ethanol. Bio-guided assay was selected as an evaluation method to further fractionate radioprotective component from *P. koraiensis* bark extract. Total phenolic and flavonoid contents in fractions were also measured. Rat splenocytes were prepared by using mechanical trituration method. DNA damage was assessed as comet parameters (tail DNA%, tail length, tail moment, olive tail moment). The levels of malondialdehyde (MDA), and activity of superoxide dismutase (SOD), catalase (CAT) in cultured rat splenocytes were also measured. **Results:** The radioprotective effects decreased from rutin >95% ethanol extracts of *Pinus koraiensis* bark (95EEP) >40AEP > WEP. The stimulating effects decreased from rutin > n-butanol extract (NBE) > EAE. The results demonstrate that there exists toxic ingredients (PEE and dichloromethane extract), proliferative-promoting, radioprotective component (EAE and NBE) in 95EEP. fraction eluted from n-butanol fractions of 95EEP with 50% methanol solution (NBEPKB-50ME), a fraction of NBE result from bio-guided isolation, demonstrates good radioprotective efficacy on rat splenocytes. NBEPKB-50ME pretreated rat splenocytes demonstrated progressively reduced levels of MDA when compared with γ-ray exposed cells. Different dose of NBEPKB-50ME pretreatment with 8 Gy-irration showed an increase in enzymatic antioxidant. **Conclusions:** Proliferative-promoting efficacy, radioprotective effect of different solvents extracts of the bark of *P. koraiensis* were investigated in this work. NBEPKB-50ME was the best elution in NBE, especially in restoring SOD, CAT activities, content of GSH, decreasing DNA damage.

Key words: Fractionation, *Pinus koraiensis* bark, radioprotective

SUMMARY

- The radioprotective effects decreased from rutin > 95EEP > 40AEP > WEP. The extract of Petroleum ether, dichloromethane extract (DME) of 95% ethanol extract of *P. koraiensis* (PEE, DME) show toxic effect on rat splenocytes. The extract of Ethyl acetate, n-butanol extract of 95% ethanol extract of *P. koraiensis* (EAE, NBE) show proliferative-promoting, radioprotective effect on rat splenocytes
- Single-cell gel electrophoresis was used to evaluate the spleen cell DNA damage parameters affected by gamma-radiation and addition of best component NBEPKB-50Me from extract of *P. koraiensis* bark
- NBEPKB-50ME pretreatment with 8 Gy-irradiation showed an increase in enzymatic antioxidant capacity. NBEPKB-50ME pretreated (80, 160, 320, 480 μg/ml) rat splenocytes demonstrated progressively reduced levels of MDA when compared with γ-ray exposed cells.



Abbreviations used: MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; PEE: Petroleum ether Extract; DME: Dichloromethane extract; EAE: Ethyl acetate extract; NBE: n-butanol extract; WAP: Water extracts of *Pinus koraiensis* bark; 40AEP: 40% acetone extracts of *Pinus koraiensis* bark; 95EEP: 95% ethanol extracts of *Pinus koraiensis* bark; TPC: Total phenolic content; TFC: Total flavonoid content; NBEPKB-50ME: Fraction eluted from n-Butanol fractions of 95EEP with 50% methanol solution.

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DOI: 10.4103/pm.pm_409_16

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INTRODUCTION

Radiation protection is a long-term research project since X-ray was found in 1901.[1] Radiation can induce DNA damage to cell, decline of cell viability, decease of activity of antioxidant enzymes. Radiation protection experts are constantly looking for natural anti-radiation agent due to the side effect of synthetic anti-radiation agent, such as amifostine.[2] So natural medicinal chemists have been looking for

Cite this article as: Yun K, Bai JH, Wang Z. *In Vitro* bioassay-guided isolation of radioprotective fractions from extracts of *Pinus koraiensis* bark. Phcog Mag 2017;13:712-8.

radiation protectant from natural products for many years. Several natural antioxidants have radioprotective effect *in vitro* and/or *in vivo*, such as soy isoflavone,[3] procyanidins from grape seeds,[4] *Coleus aromaticus* extract,[5] *Mentha piperita* (Linn.),[6] lymphocytes and/or lymphoid organs is susceptible to be insulted by γ-radiation, and lymphocytes survival rate is deemed as a good indications to screen natural products which may render radioprotective effect, single-cell gel electrophoresis, which is a commonly toxicological method, was selected to determine DNA damage caused by nuisance factor. Thus this method is also used to evaluate the extent of DNA damage induced by γ-radiation.

In previous study, Li *et al*. reported that "Pine polyphenols of *Pinus koraiensis* inhibit damages insulted by radiation in mice,"[7] the mixture of pine polyphenols was purified from cone of *P. koraiensis*. Yun and Wang reported "Antioxidant activities of poly phenols from *P. koraiensis in vitro*," the extract was prepared from *P. koraiensis* bark.[8] This fact hint that *P. koraiensis* bark extract may also possess radioprotective efficacy.

So, the purpose of this work was to evaluate total phenolics content, total flavonoids content, proliferation-promoting efficacy of different extracts of *P. koraiensis* bark. We analyzed the relationships between total phenolics, flavonoid content, and splenocytes cytotoxicity/proliferation/radioprotection by MTT assay. The effect of *P. koraiensis* bark extract on DNA damage, cell malondialdehyde (MDA), cellular antioxidant status in rat splenocytes insulted by gamma radiation were also assessed.

MATERIALS AND METHODS

Chemicals and reagents

EDTA disodium salt (EDTA-2Na), and Tris-HCl buffer (16 mM, pH 8.0) were purchased from Sigma (St. Louis, MO, USA). The water used in the study was purified with a Simplicity 185 Personal water purification system (Millipore, Bedford, MA, USA). Folin-ciocalteu reagent, gallic acid, trichloroacetic acid, sodium sarcosinate, were of the highest commercial grade (Tianjin Bodi Chemical Reagent, China). Normal melting point agarose and low melting point (promega, USA). MDA assay kit (colorimetric method), total superoxide dismutase (T-SOD) assay kit (hydroxylamine method), catalase (CAT) assay kit (visible light) (Nanjing Jiancheng Bioengineering Institute).

Sample extraction

The dried *P. koraiensis* bark (5000 g) was ground and extracted with water 40% acetone, 95% ethanol, labeled WEP, 40AEP, and 95% ethanol extracts of *Pinus koraiensis* bark (95EEP), respectively labeled 95EEP. Extract proves complied with the procedure previous reported. The *P. koraiensis* bark extract was fractionated by petroleum ether, dichloromethane, ethyl acetate and n-butanol. The resultant fractions will continue to be re-fractioned by silicon gel or octadecylsilyl (ODS) or sephadex according to the outcome of the experiment.

Determination of the total phenolic content

Total phenolic content (TPC) was determined by reported method with slight modifications.[9]

Determination of total flavonoid content

Total flavonoid content was measured using a previously described method with some modifications.[10]

Radioprotective effect of *Pinus koraiensis* bark extract on splenocytes insulted by γ-ray

Splenocytes were pretreated with *P. koraiensis* bark extracts in 96 plates, 2 h later. The 96 plates were received 8 Gy γ-ray at a dose rate of 1.33 Gy/min (Maize Research Institute of Heilongjiang Academy of Agricultural Sciences). After 20 h, MTT in phosphate-buffered saline were put into each well. Cell viability affected by *P. koraiensis* bark extract and γ-ray was calculated as previously reported.

Proliferative/cytotoxic effects of extract on splenocytes

Experimental animals for primary splenocytes cultures

Primary splenocytes were prepared according to the procedure previously reported. This study was approved by the Ethics Committee of Institutional Research Board of Harbin Medical University for animal studies and was performed in line with the Guiding principles for the Care and Use of Laboratory Animals. The viability affected by *P. koraiensis* bark extract and/or γ-ray was assessed by MTT assay as previously reported.

Study design

To characterize the radioprotective of *P. koraiensis* bark extract on splenocytes viability splenocytes were treated as follows: (I) Normal (pretreatment with solvent); (II) irradiation (pretreatment with solvent); (III).γ-ray + rutin (0–640 μg/ml); (IV).γ-ray + 40AEP (0–640 μg/ml); (V) γ-ray + 95EEP (0–640 μg/ml); (VI) γ-ray + WEP (0–640 μg/ml).

To characterize the effect of fractions fractionated by different polar organic solvents and/or separated from Different types of column chromatography from *P. koraiensis* bark extract on splenocytes proliferation, splenocytes were treated as follows: (I) Normal (pretreatment with solvent); (II) rutin (0–640 μg/ml); (III) fraction 1; (IV) fraction 2; (V) fractions 3….

Comet assay

The effect of *P. koraiensis* bark extract on DNA damage insulted by gamma radiation in rat splenocytes was proceeded by single cell gel electrophoresis describe by Abt *et al*.[11,12] with minor modifications. Rat splenocytes (1×10^6 cells) were pretreated with different concentrations of *P. koraiensis* bark extract (80, 160, 320 μg/ml) for1 h and then placed to receive 8, 10 Gy radiation. Slides were embedded with 200 μl of 1% normal melting agarose, and put into freezer overnight to get Slide A. 10^5 cells was mixed with 200 μl of 0.5% low melting agarose (LMA) at 37°C, the mixture was blowed out with a pipette on Slide A to obtain Slide B. The Slides B were covered by 0.5% LMA to made as slide C. Slide C was immersed in ice-cold lysis solution (2.5M sodium chloride, 100 mM ethylenediamine tetraacetic acid disodium salt, 1% Triton X-100, 1% dimethyl sulphoxide, 10 mM Tris-HCl, and 1% sodium sarcosinate, pH 10) for 60 min at 4°C. Denaturation was carried out in alkaline buffer (300 mM sodium hydroxide, 1 mM Na_2-EDTA and 0.2% dimethyl sulphoxide, pH 13.0) for one-third hour. Electrophoresis was conducted at 25 V for one-third hour. All the procedure were performed under yellow light to inhibit additional DNA damage. Following electrophoresis, the slides C were gently rinsed with 0.4M Tris-HCl buffer, pH 7.4. The slides C were dyed with 50 μl of Ethidium bromide (20 μg/ml) and analyzed using a Fluorescent microscope (Nikon).[13]

The images (60–100 cells/slide) were captured and analysis of the content of DNA damage was performed using CASP software (Beijing Biolaunching Technologies Co., Ltd.Beijing. P.R.China) by which percent DNA in the tail (% tail DNA), tail moment, tail length, and

olive tail moment can be got. Data are the average of three independent experiments.[11,12,14]

In vitro studies on effect of the best fraction obtained by bio-guided assay: Lipid peroxidaton and cellular antioxidant status

Radiation induced malondialdehyde in rat splenocytes

Rat splenocytes exposed to γ-ray could result in lipid peroxidaton, which usually was measured in terms of nmoles of MDA/mg protein, this assay was performed with cell MDA assay kit.

Radiation induced decrease of cellular antioxidant status in rat splenocytes

Rat splenocytes exposed to γ-ray could result in decrease of SOD activities, CAT activities in rat splenocytes, these assays were measured by T-SOD assay kit, CAT assay kit respectively.

Statistical analysis

All data are expressed as the means ± standard deviation of three replicates. Statistical analyses were performed using OriginPro Version 8.5 software (OriginLab Corporation, Northampton, MA, USA). Pearson's correlation coefficients and one-way analysis of variance were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) to identify differences between samples; $P < 0.05$ was considered statistically significant.[15]

RESULTS

Radioprotective effects of extract of bark of *P. koraiensis* and rutin on rat splenocytes insulted by 8 Gy γ-ray.

Radioprotective effects of extract of bark of *P. koraiensis* and rutin on rat splenocytes insulted by 8 Gy γ-ray were assessed. The results showed that the 40AEP, 95EEP, and rutin increased splenocytes viability at concentrations of 0–320 μg/ml and inhibited proliferation at concentrations of >320–640 μg/ml [Figure 1]. The splenocytes viability was 122.68% ±5.68% with 320 μg/ml 95EEP; compared to WEP, 40AEP had significant ($P < 0.05$) radioprotective effect and 95EEP had significant ($P < 0.05$) cytotoxic effects at concentrations up to 640 μg/ml, and exhibited slightly lower stimulatory effects than did rutin. The reference, rutin, had a significant ($P < 0.05$) radioprotective effect at concentrations up to 640 μg/ml. The radioprotective effects decreased from rutin > 95EEP > 40AEP > WEP. All the trend of this assay is similar to the previous analysis of viability. The radioprotective effects of extract of bark of *P. koraiensis* and rutin on rat splenocytes also strongly positively correlated with phenolic and flavonoid contents at the concentration range between 20 and 320 μg/ml ($r = 0.961$ and $P = 0.002$ for 40AEP; $r = 0.989$ and $P < 0.001$for 95EEP; and $r = 0.919$ and $P = 0.003$ for rutin) at concentrations below 640 μg/ml.

Outcome of bioassay-guided fractionation

In view of the proliferative, radioprotective effect of three solvents of *P. koraiensis* bark extract. The data insinuate us that proliferation

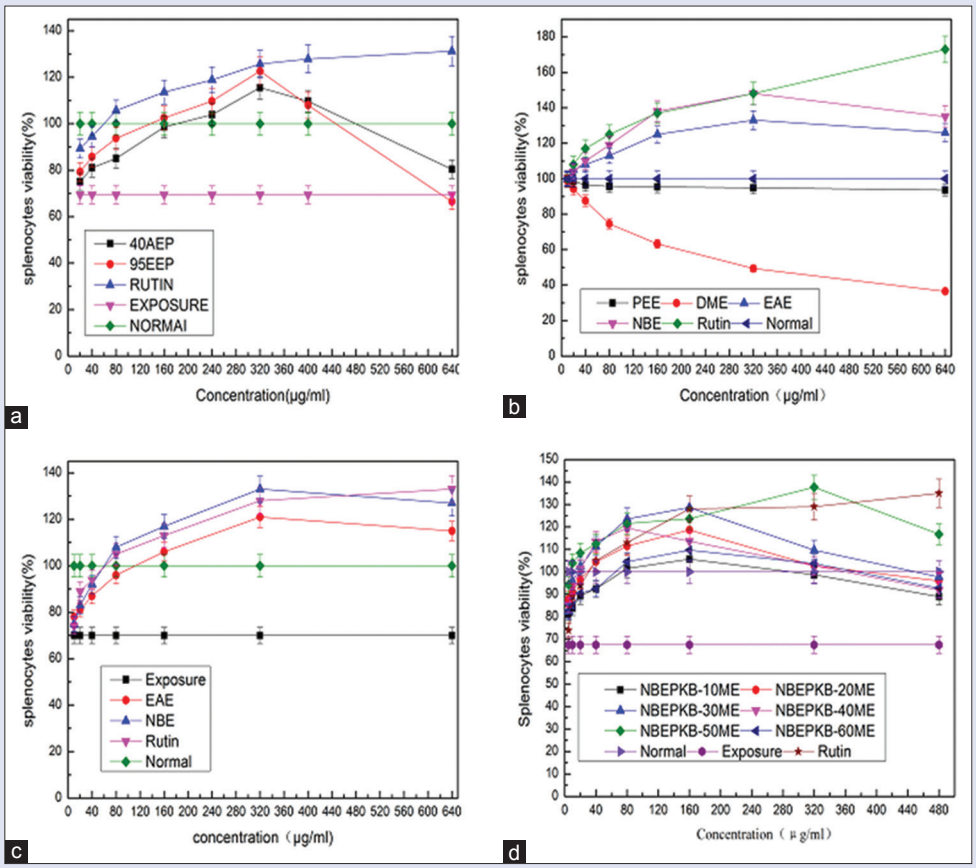

Figure 1: Splenocytes viability affected by three different solvent extract of bark of *Pinus koraiensis*, fractions of 95% ethanol extracts of *Pinus koraiensis* bark, rutin and 8 Gy γ-ray. (a) Radioprotective effects of three different solvent extract of bark of *Pinus koraiensis* and rutin on rat splenocytes insulted by 8 Gy γ-ray. (b) Splenocytes viability affected by fractions of 95% ethanol extracts of *Pinus koraiensis* bark and rutin. (c) Radioprotective effects of fractions of 95% ethanol extracts of *Pinus koraiensis* bark and rutin on rat splenocytes insulted by 8 Gy γ-ray. (d) Radioprotective effects of fractions eluted from N-butanol extract with methanol solution of different concentrations and rutin on rat splenocytes insulted by 8 Gy γ-ray

efficacy of 95EEP was better than that of 40AEP and water extracts of *Pinus koraiensis* bark (result of previously study), there may be simultaneously exist proliferative fraction and cytotoxic faction. So, it is necessary to fractionate these two fractions. So, we extracted more raw materials in order to ensure that the separation of the fractions were sufficient to be separated. The crude *P. koraiensis* bark extract was prepared in 95% ethanol 1:7 (w/v, 5 kg/35 L), concentrated by using Rotary evaporator to dryness (328.37 g), obtained extract was uniformly dispersed in water to get aqueous suspension and partitioned with organic solvents follow the sequence: Petroleum ether extract (PEE), dichloromethane extract (DME), ethyl acetate extract (EAE), n-butanol extract (NBE).[16]

Component which having a better proliferation-promoting effects would be continuously separated to obtain best active ingredient. If the result prompted us to separate low polar fraction (DME, EAE), the silica gel column chromatograph would be employed by eluting with proportions of solvent as far as (DME) was concerned. PEE and EAE were preferred. In terms of the separation of high polar fraction (NBE), the ODS gel column chromatograph would be employed by eluting with proportions of solvent (methanol and water).[17]

Total phenolic and flavonoid contents in fractions

The TPCs of four fractions of 95EEP are listed in Table 1, and the values are derived from the regression equation as previously reported and expressed in gallic equivalents. The flavonoid contents of four fractions of 95EEP are listed in Table 1; The phenolic and flavonoid contents were higher in the NBE than in the EAE, DME and PEE. The differences in total polyphenolic compound, flavonoid contents can be attributed to polarity of different solvent. The results prove that the separation is an indispensable mean to increase content of polyphenols in fractions.

Splenocytes viability affected by fractions of 95% ethanol extracts of *Pinus koraiensis* bark and rutin

Splenocytes viability affected by fractions of 95EEP was assessed [Figure 1]. The results showed that PEE and DME decreased splenocytes viability at concentrations of 0–640 μg/ml. This phenomenon indicates that these fractions are cytotoxic [Figure 1]. EAE and NBE increased splenocytes viability at concentrations of 0–320 μg/ml. The stimulatory effect was 148.19% ± 5.88% with 320 μg/ml NBE; compared to EAE, NBE had slightly inhibitory effects at concentrations between 320 and 640 μg/ml, and still exhibited stimulatory effects than did Ethyl acetate fraction. The stimulating effects decreased from rutin > NBE > EAE. The results demonstrate that there exist toxic ingredients in 95EEP. The stimulation of rat splenocytes proliferation also strongly positively correlated with phenolic and flavonoid contents at concentration range between 10 and 640 μg/ml (r = 0. 910 and P = 0.004for EAE; r = 0.885 and P = 0.008 for NBE).

Table 1: Total phenolic, total flavonoids content of fractions of 95% ethanol extracts of *Pinus koraiensis bark*

Fractions	Total phenolic (gallic acid equivalent) (mg/g)	Total flavonoid (rutin equivalent) (mg/g)
Petroleum ether fraction	5.629±0.279	4.413±0.213
Dichloromethane fraction	63.871±2.275	39.935±1.928
Ethyl acetate fraction	437.837±22.21	228.762±11.049
n-butanol fraction	773.965±40.571	567.357±27.403

Radioprotective effects of fractions of 95% ethanol extracts of *Pinus koraiensis* bark and rutin on rat splenocytes insulted by 8 Gy γ-ray

Radioprotective effects of fractions of 95EEP on rat splenocytes insulted by 8 Gy γ-ray were assessed. The results showed that ethyl acetate fraction, n-butanol fraction, and rutin increased splenocytes viability at concentrations of 0–320 μg/ml and inhibited proliferation at concentrations of >320–640 μg/ml [Figure 1]. At concentrations range 5–320 μg/ml, significant difference (P < 0.05) was found in the radioprotective effect on splenocytes viability affected by EAE and NBE. The splenocytes viability was 130.02% ± 6.32% with 320 μg/ml NBE; compared to EAE and rutin had significant (P < 0.05) stimulatory effects at concentrations range between 40 and 640 μg/ml, the radioprotective effects decreased from NBE > rutin > EAE. The radioprotective effects of fractions of 95EEP and rutin on rat splenocytes also strongly positively correlated with phenolic and flavonoid contents (r = 0.893 and P = 0.007 for EAE; r = 0.825 and P = 0.022 for NBE) at concentrations below 640 μg/ml.

Splenocytes proliferation assay demonstrated NBE is the best solvent extract, given the principles of proliferative-promoting activity-oriented, NBE was subjected to separation by using ODS column chromatography with methanol solution of different concentrations. Meanwhile, the elution was respectively named NBEPKB-10ME, NBEPKB-20ME, NBEPKB-30ME, NBEPKB-40ME, fraction eluted from n-Butanol fractions of 95EEP with 50% methanol solution (NBEPKB-50ME), NBEPKB-60ME, according to the eluent used. All results are described below.

Radioprotective effects of fractions eluted from N-butanol extract with methanol solution of different concentrations and rutin on rat splenocytes insulted by 8 Gy γ-ray

Radioprotective effects of fractions eluted from n-butanol fraction with Methanol solution of different concentrations on rat splenocytes insulted by 8 Gyγ-ray were assessed [Figure 1]. The results showed that NBEPKB-10ME, NBEPKB-20ME, NBEPKB-30ME, NBEPKB-40ME, NBEPKB-60ME, increased splenocytes viability at concentrations of 5–160 μg/ml and inhibited proliferation at concentrations of >320–640 μg/ml, NBEPKB-50ME, increased splenocytes viability at concentrations of 5–320 μg/ml [Figure 1]. At concentrations of 5 μg/ml, significant difference (P < 0.05) was found in the radioprotective effect on splenocytes viability affected by NBEPKB-50ME and NBEPKB-10ME, NBEPKB-60ME, rutin.

Effect of NBEPKB-50Me on γ-ray induced DNA damage

The radiation dose of 8 Gy caused a considerable increase of cellular DNA insult in rat splenocytes. The mean value of comet parameters (tail DNA%, tail length, tail moment, olive tail moment) were detected to be increased in all group treated by γ-ray. In rat splenocytes, tail DNA% was increased from 3.24 ± 0.48 (0 Gy) to 32.35 ± 4.36 (8 Gy) and 38.91 ± 4.78 (10 Gy), Tail length from 6.00 ± 0.89 (0 Gy) to 37 ± 5.36 (8 Gy) and 39.16 ± 5.19 (10 Gy), tail moment from 0.54 ± 0.07 (0 Gy) to 7.96 ± 1.01 (8 Gy) and 9.49 ± 1.28 (10 Gy), olive tail moment from 0.83 ± 0.11 (0 Gy) to 8.46 ± 1.12 (8 Gy) and 10.10 ± 1.34 (10 Gy). When 80 μg/ml NBEPKB-50ME was added to the medium of rat splenocytes 1 h before 8 Gy radiation, an decrease in the comet parameters such as tail DNA %, tail length, tail moment, olive tail moment were reduced to the levels of 29.34 ± 3.77, 32.16 ± 4.07, 6.81 ± 0.94, 7.76 ± 1.05. When dose was added to 160 μg/ml, comet parameters as described above were reduced to the levels of 27.36 ± 3.78, 24.66 ± 3.26, 5.82 ± 0.79,

6.77 ± 0.91, when dose was added to 320 μg/ml, comet parameters as described above were reduced to the levels of 14.10 ± 2.04, 18.5 ± 2.25, 4.12 ± 0.57, 4.86 ± 0.65. When 320 μg/ml NBEPKB-50ME was added, comet parameters as described above were reduced to the levels of 16.25 ± 2.22, 22.16 ± 2.92, 18.5 ± 2.25, 4.51 ± 0.60, 5.20 ± 0.64.

Similarity in 10 Gy γ-ray exposed rat splenocytes administered with different dose of NBEPKB-50ME, tail length, tail DNA%, tail moment, tail olive moment were reduced to levels of 34.33 ± 4.54, 35.37 ± 4.51, 8.15 ± 1.09, 9.22 ± 1.20, respectively, at a concentration of 80 μg/ml, 30.5 ± 4.03, 32.10 ± 4.27, 6.92 ± 0.93, 7.99 ± 1.04, respectively, at a concentration of 160 μg/ml, 23.66 ± 2.25, 17.03 ± 2.15, 4.91 ± 0.68, 5.80 ± 0.84, respectively, at a concentration of 320 μg/ml. As to rutin, comet parameters as described above were reduced to the levels of 26.5 ± 3.14, 19.41 ± 2.64, 5.27 ± 0.73, 6.14 ± 0.82; comet parameters are shown in Figures 2 and 3.

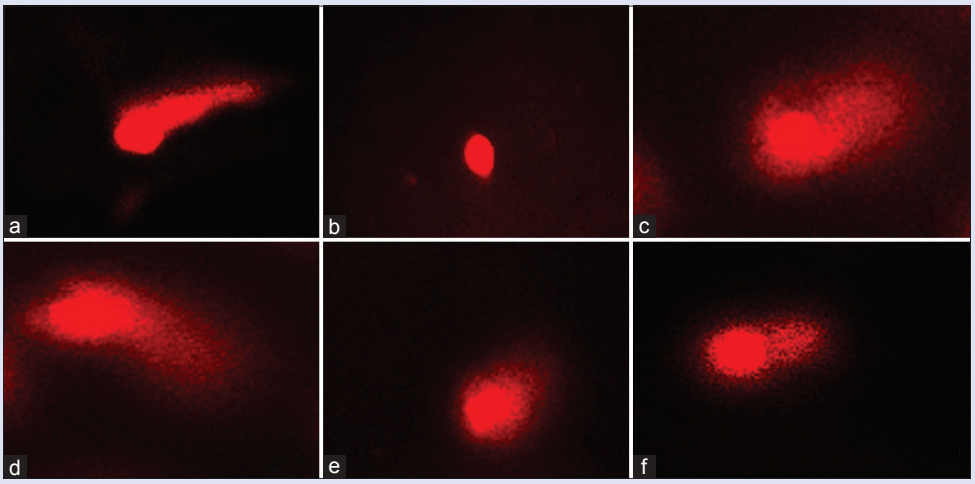

Figure 2: Effect of NBPKB-50ME on DNA damage in rat splenocytes exposed to 10 Gy γ radiation as detected by comet assay. (a) Exposed to 10 Gy. (b) Normal. (c) Exposure + NBEPKB-50ME 80 μg/ml. (d) Exposure + NBEPKB-50ME 160 μg/ml. (e) Exposure + NBEPKB-50ME 320 μg/ml. (f) Exposure + rutin 320 μg/ml

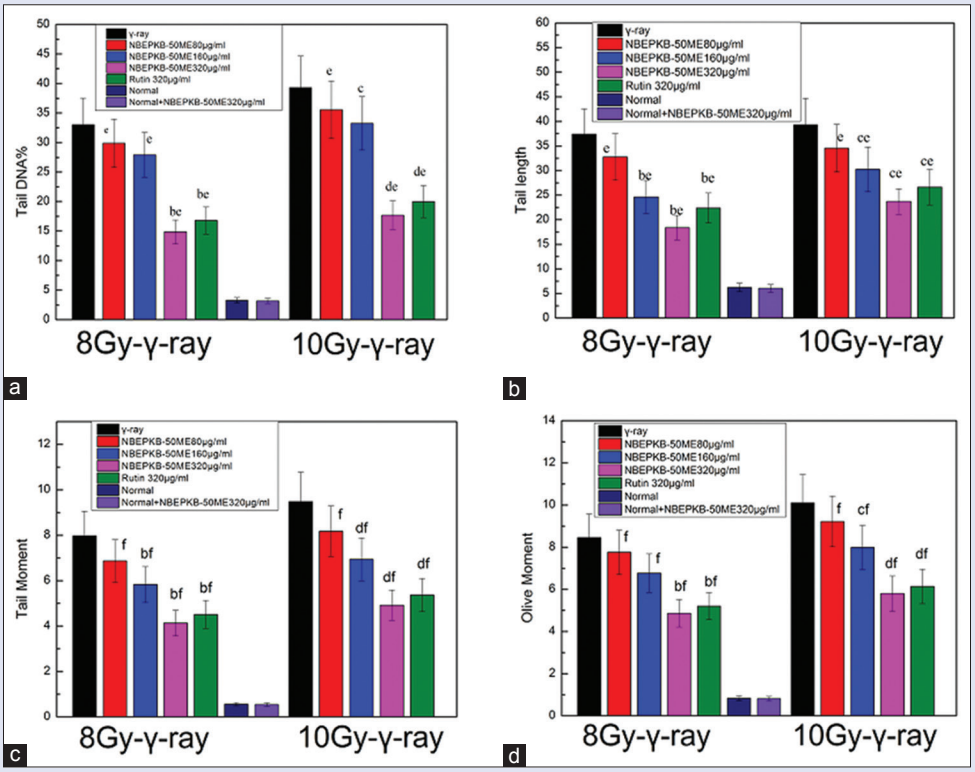

Figure 3: Effect of NBPKB-50ME on DNA damage in rat splenocytes exposed to 8, 10 Gy γ radiation as detected by comet assay analyzed by CASP software. (a) Tail DNA%. (b) Tail length. (c) Tail moment. (d) Tail olive moment. Values are ± standard deviation of two experiment conducted in triplicate. [a]$P<0.001$, [b]$P<0.01$, as compared to 8 Gy irradiated control (Bonferroni test), [c]$P<0.05$, [d]$P<0.01$, as compared to 10 Gy irradiated control (Bonferroni test), [e]$P<0.05$, [f]$P<0.01$, as compared to normal (Bonferroni test)

Effect of 95% ethanol extracts of *Pinus koraiensis* bark with 50% methanol solution on the levels of malondialdehyde, and activity of superoxide dismutase, catalase in cultured rat splenocytes when exposed to gama-ray

The concentration of MDA increased dramatically in rat splenocytes when exposed to gama-ray [Figure 4]. In this assay, NBEPKB-50ME pretreated (80, 160, 320, 480 μg/ml) rat splenocytes demonstrated progressively reduced levels of MDA when compared with γ-ray exposed cells. Figure 4 depict activities of enzymatic antioxidant SOD. CAT in rat splenocytes in this assay, γ-ray alone treated groups demonstrated a dose-dependent reduced in enzymatic status of rat splenocytes whereas different dose of NBEPKB-50ME pretreatment with 8 Gy-irration showed an increase in enzymatic antioxidant, but NBEPKB-50ME pretreatment could not restore the enzymatic antioxidant to near normal.[18]

DISCUSSION

It is well known that main damage caused by gama-ray to living cells are aqueous free radicals. As to radioltic product of water, eaq, •OH and •H usually react with oxygen and bring out various reactive oxygen species, Fe^{2+} and Cu^{2+} can react with •OH generated by radiolysis of water, spleen is a radio-sensitive organ may be partly due to that spleen is just the organ insulted by iron-mediated Fenton reaction triggered by ferritin from aged red cell which are swallowed by phagocytes.[19-21]

Several reports proved that radioprotective effect of polyphenols, such as, polyphenols from *Phyllanthus amarus* Linn,[22] curcumin,[23] quercetin.[18] Thus, that extracts of *P. koraiensis* bark having capacity of increasing splenocytes viability could be deemed as an indicator of radioprotective potential. The outcome of bio-guided assay proved that toxic fractions are PEE and DME, cellular proliferative-promoting/radioprotective fractions are EAE and NBE. Further track by ODS column chromatography help us succeed in finding NBEPKB-50ME.

Ionizing radiation is an effective inducer of cell death and trigger of DNA damage. The main target insulted by irradiation is genomic DNA in the living cells. The type of DNA damage include: Double single-strand breaks, single-strand breaks, base damage, deoxyribose damage, Jagetia.[24] The radioprotective efficacy of NBEPKB-50ME on consequent that double strand break induced by 8 Gy, 10 Gy γ-ray was evaluated by Single cell gel electrophoresis. MDA is the product of lipid peroxidation induced by radiation. There has been a sharp rise of MDA after exposed in γ-ray in rat splenocytes, this trend declined because of the administration of NBEPKB-50ME in the splenocytes medium. In contrast, there has been a sharp decline of SOD, CAT, after exposed in γ-ray in rat splenocytes, this trend rised because of the same treatment.

CONCLUSIONS

Proliferative-promoting efficacy, radioprotective effect of different solvents extracts of the bark of *P. koraiensis* was investigated in this work. The difference outcome resulted from Primary separation hint us to make a further probe by bio-guided assay, NBEPKB-50ME was the best elution in NBE, especially in restoring SOD, CAT activities, decreasing DNA damage, all of which induced by γ-ray. Our results suggest the bark of *P. koraiensis* and the best radioprotective component still need to be obtained by isolation. Toxic component (PEE and DME) were need to be identified in the next work. However, this study was performed exclusively *in vitro*. Additional *in vivo* experiments maybe lead to a more comprehensive understanding of the radioprotective potential of *P. koraiensis* bark.

Acknowledgements

This work has been financially supported by the National Natural Science Foundation of China (Grant No. 31170510) and The Chinese National Youth Fund (Grant No. 31401618).

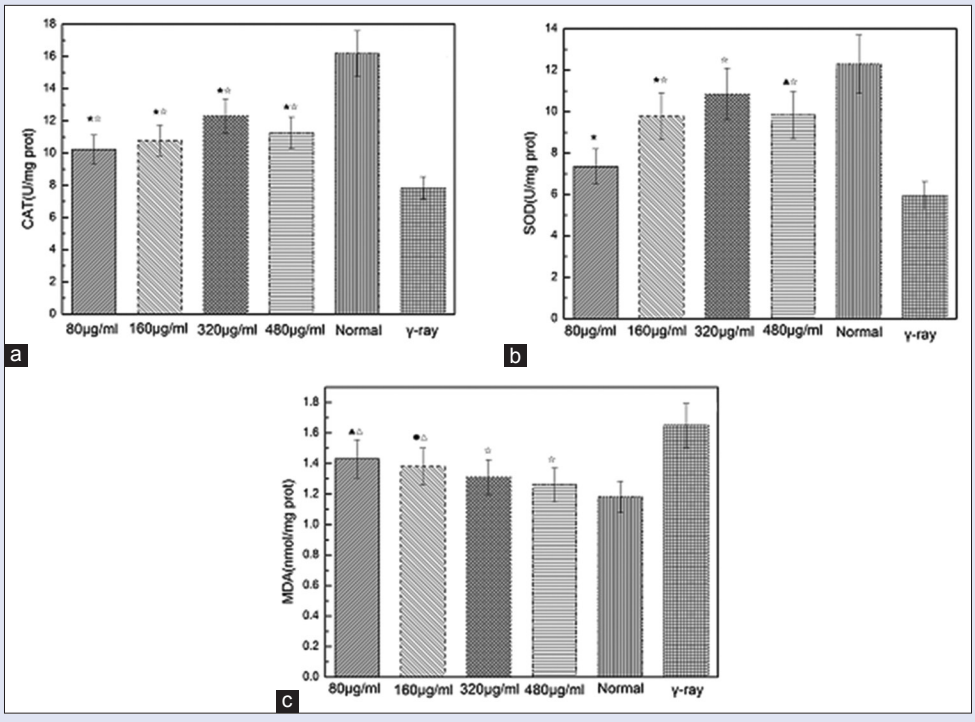

Figure 4: Effect of NBEPKB-50ME on the levels of (catalase, CAT) (a), (superoxide dismutase, SOD) (b), (malondialdehyde,MDA) (c) in cultured rat splenocytes when exposed to γ-ray. •P<0.05, ▲P<0.01, ★P<0.001, as compared to normal. ◦P<0.05, △P<0.01, ☆P<0.001, as compared to γ-ray

Financial support and sponsorship

I Would like to acknowledge the two fund National Natural Science Foundation of China (Grant No. 31170510) and Chinese National Youth Fund (Grant No. 31401618) for their suppots.

Conflicts of interest

There are no conflicts of interest.

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