Study of extracts from *Poria cocos* solid-state fermented product on protection and repair of brain cells in zebrafish embryos

茯苓固態發酵產物之萃取物對斑馬魚胚胎腦細胞保護和修復的研究

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Poria cocos

http://en.wikipedia.org/wiki/File:Tuckahoe.jpg

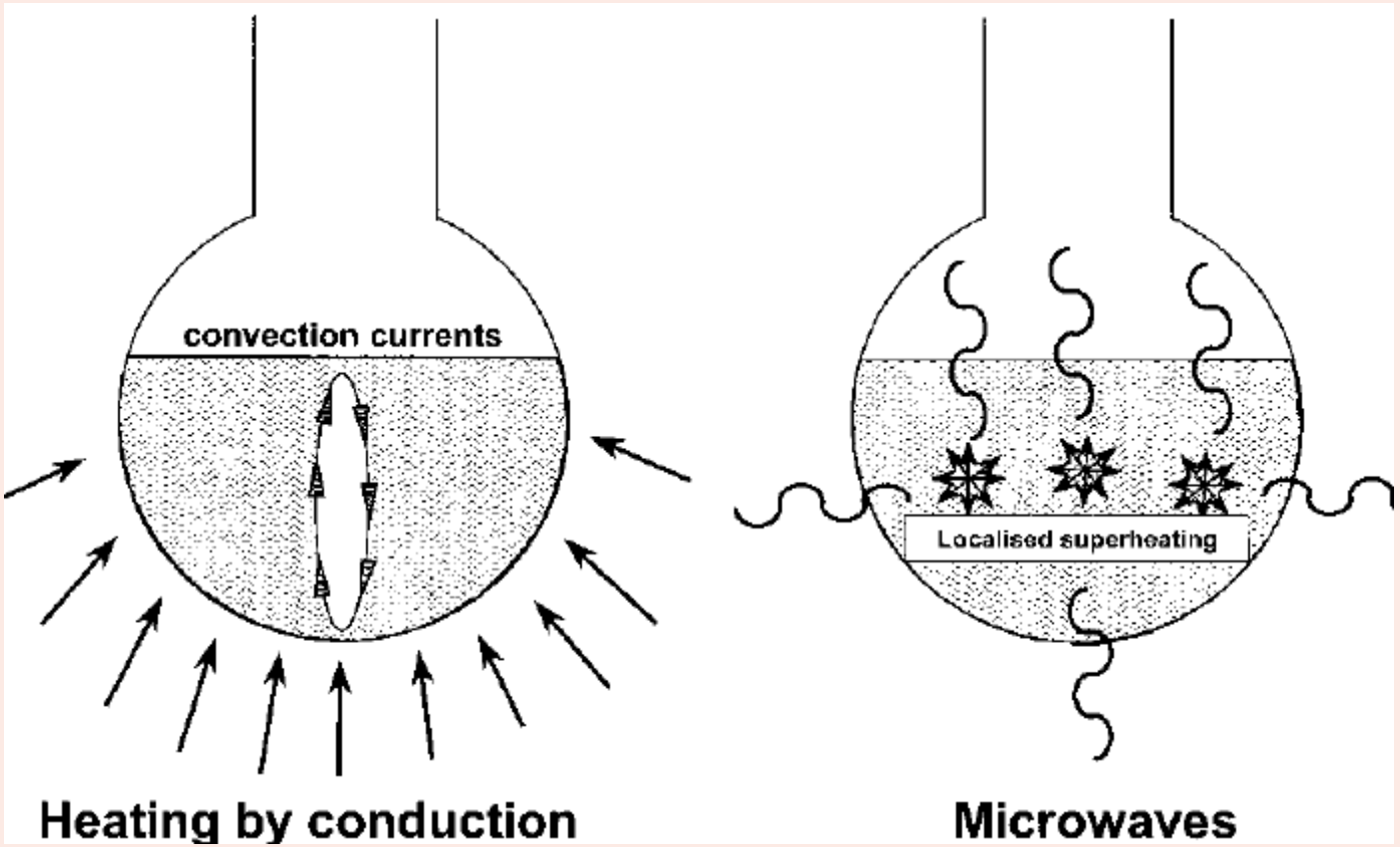
- A fungus grows on the roots of pine trees.
- The main bioactive compounds are polysaccharides and triterpenoids.
- It have bioactive functions are anti-inflammatory, antioxidant, immunomodulatory, neuroprotection, anti-tumor, diuretic, and hypoglycemic etc.

(Wang *et al.*, 2009)

Poria cocos potential in nerve cell protection

- The water extracts were promoted nPC12 cell neurite out-growth that indicate differentiation. (游，2010)
- The water extracts have a protective effect on β-amyloid 1-42 induced neuronal cell death due to its anti-oxidant properties. (Park *et al*., 2009)
- They can avoid β-amyloid-induced neuron cells death (Kanno *et al*., 2013) and reduce β-amyloid content on Alzheimer's mice of brain. (Seo *et al*., 2010)
- The extracts have neuroprotection effect of brain on ischemia-reperfusion injury mouse. (Li *et al*., 2007; Cai *et al*., 2011)

Heating mechanism of microwave extraction



(Kaufmann and Christen, 2002)

Zebrafish (*Danio rerio*)

- An excellent vertebrate model.
- Easily to maintain in large numbers and readily reproducing under laboratory conditions.
- For studying developmental biology, neurobiology, genetics research, teratology and carcinogenicity testing.

(Matthews *et al*., 2002; Reimers *et al*., 2006)

Zebrafish has transparent embryo with rapid development and easy observation.

eggs

24 hpf

36 hpf

48 hpf embryo

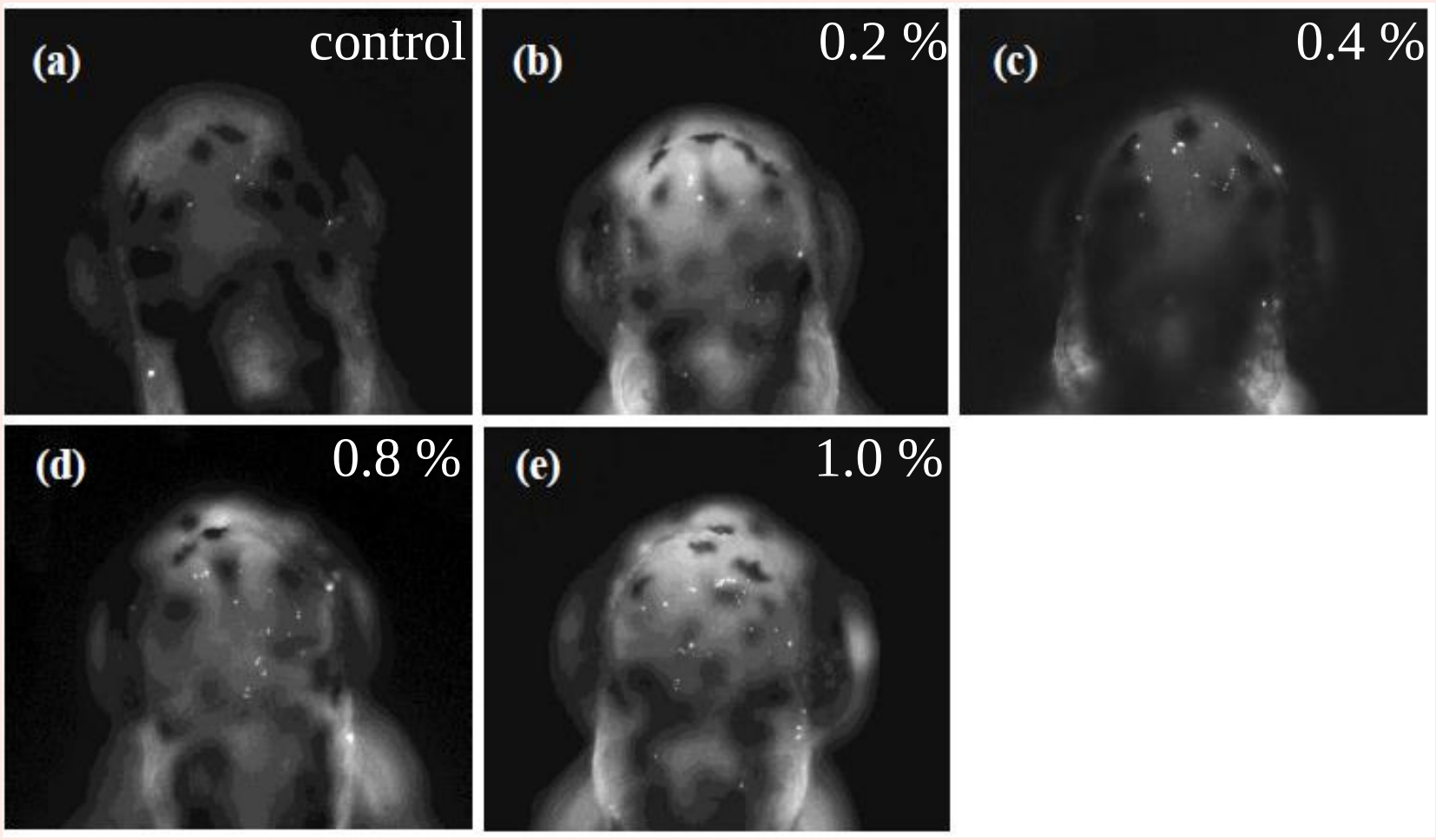


Fig. 1. Effect of different concentration ethanol: (a) control, (b) 0.2 %, (c) 0.4%, (d) 0.8%, and (e) 1% ethanol on the brain cells of zebrafish embryos.

(何，2014)

Objectives

- To study effect of water and ethanol extracts from *Poria cocos* solid-state fermented products on protection and repair of brain cells in zebrafish embryos destroyed by 1% ethanol.

Experimental design

Poria cocos (BCRC36022)

↓ pre-activation

Soybean and brown rice medium

↓

Cultivation for 0~12 weeks

↓

Sterilization

↓

Hot-air drying

↓

Grinding

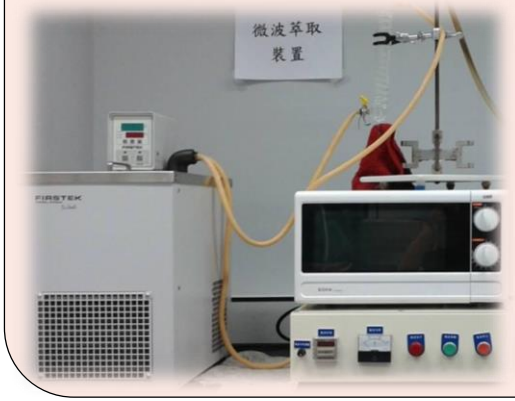
↓

Poria cocos solid-state fermented powder

↓

Microwave extraction

(300W, 5 min, solid/liquid=1:20)



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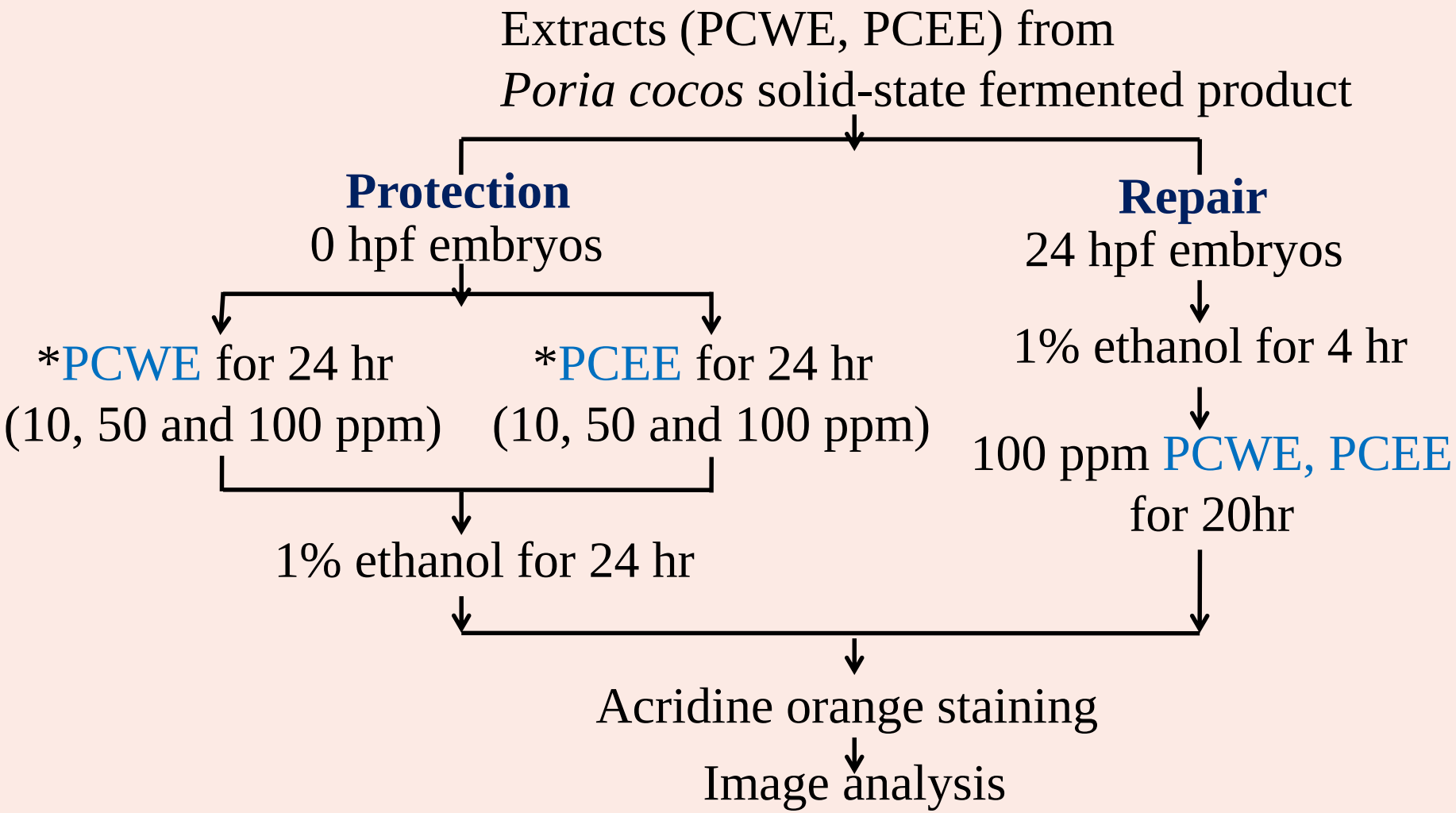
PCWE (water extract)

PCEE (ethanol extract)



*PCWE: water extracts from *Poria cocos* solid-state fermented product
*PCEE: ethanol extracts from *Poria cocos* solid-state fermented product

Results and discussion

Table 1 The change of crude polysaccharides and triterpenoids contents in soybean and brown rice mixture medium during *Poria cocos* solid-state fermentation

Time (weeks)	0	2	4	8	12
T (%)	1.03 ± 0.02^{e}	2.39 ± 0.04^{a}	1.81 ± 0.01^{d}	2.18 ± 0.09^{c}	2.29 ± 0.05^{b}
P (%)	11.94 ± 0.25^{b}	26.82 ± 0.28^{a}	21.68 ± 0.24^{c}	16.96 ± 0.14^{d}	13.26 ± 0.18^{e}

Data are expressed as mean ± S.D. (n=3). *P: crude polysaccharides; T: crude triterpenoids a-c Mean ± SD (n = 3) with different letters were significantly different in the different cultivation time ($P < 0.05$).

Table 2 Effect of ethanol and water microwave extraction order on extraction ratio (%) of *Poria cocos* fruiting body and solid-state fermented products

Poria cocos	Extraction solvent	Extraction ratio (%)	P* in water extract (%)	T* in ethanol extract (%)
Fermented product	Water	29.12±1.12	92.10	-
	Ethanol	17.81±0.84	-	13.40
Fruiting body	Water	2.71±0.35	5.54	-
	Ethanol	2.38±0.38	-	92.85

Data are expressed as mean ± S.D. (n=3). *P: crude polysaccharides; T: crude triterpenoids

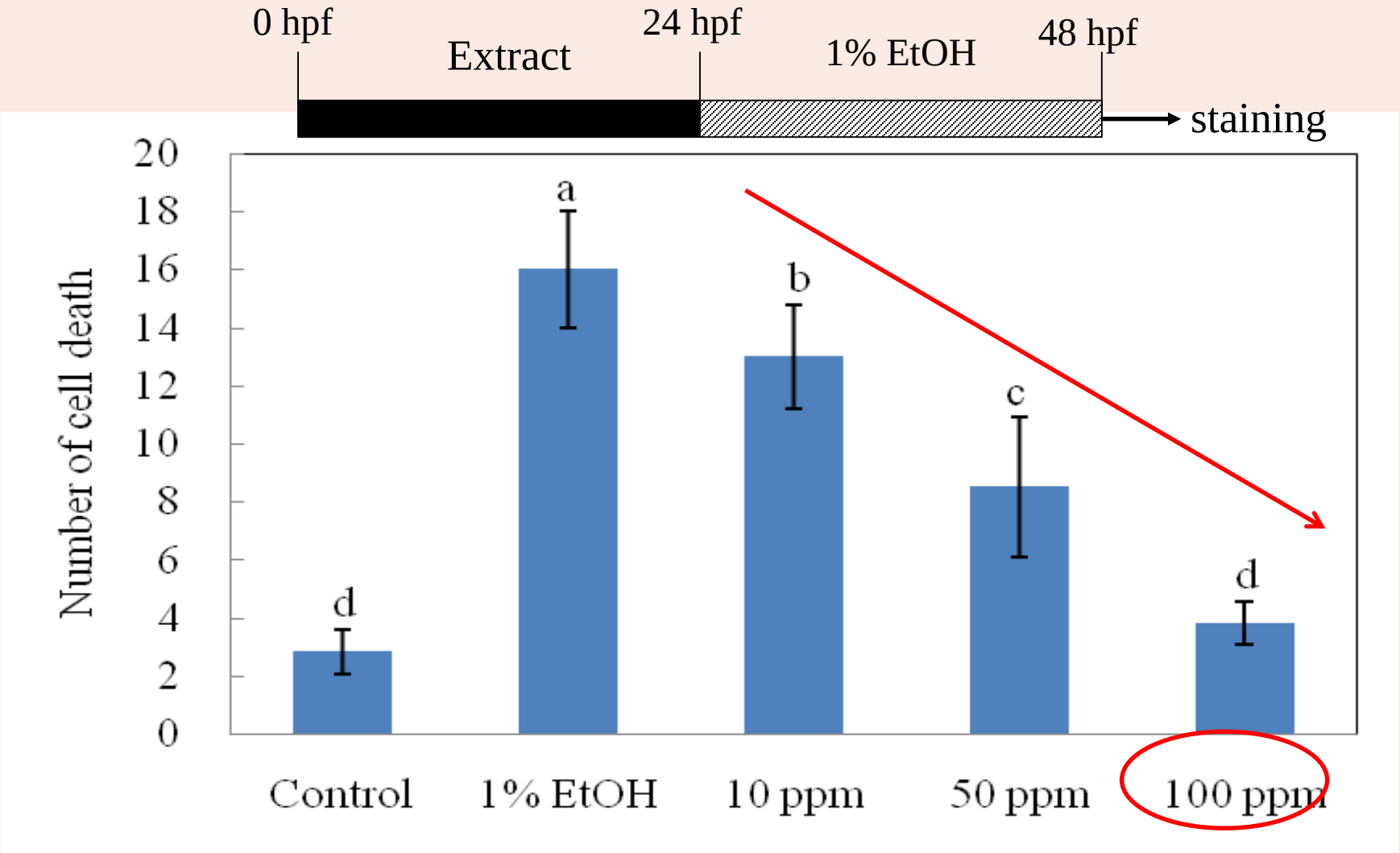


Fig. 3. Effect of protection with different concentration of PCWE on the number of died brain neuron cells in zebrafish embryo.

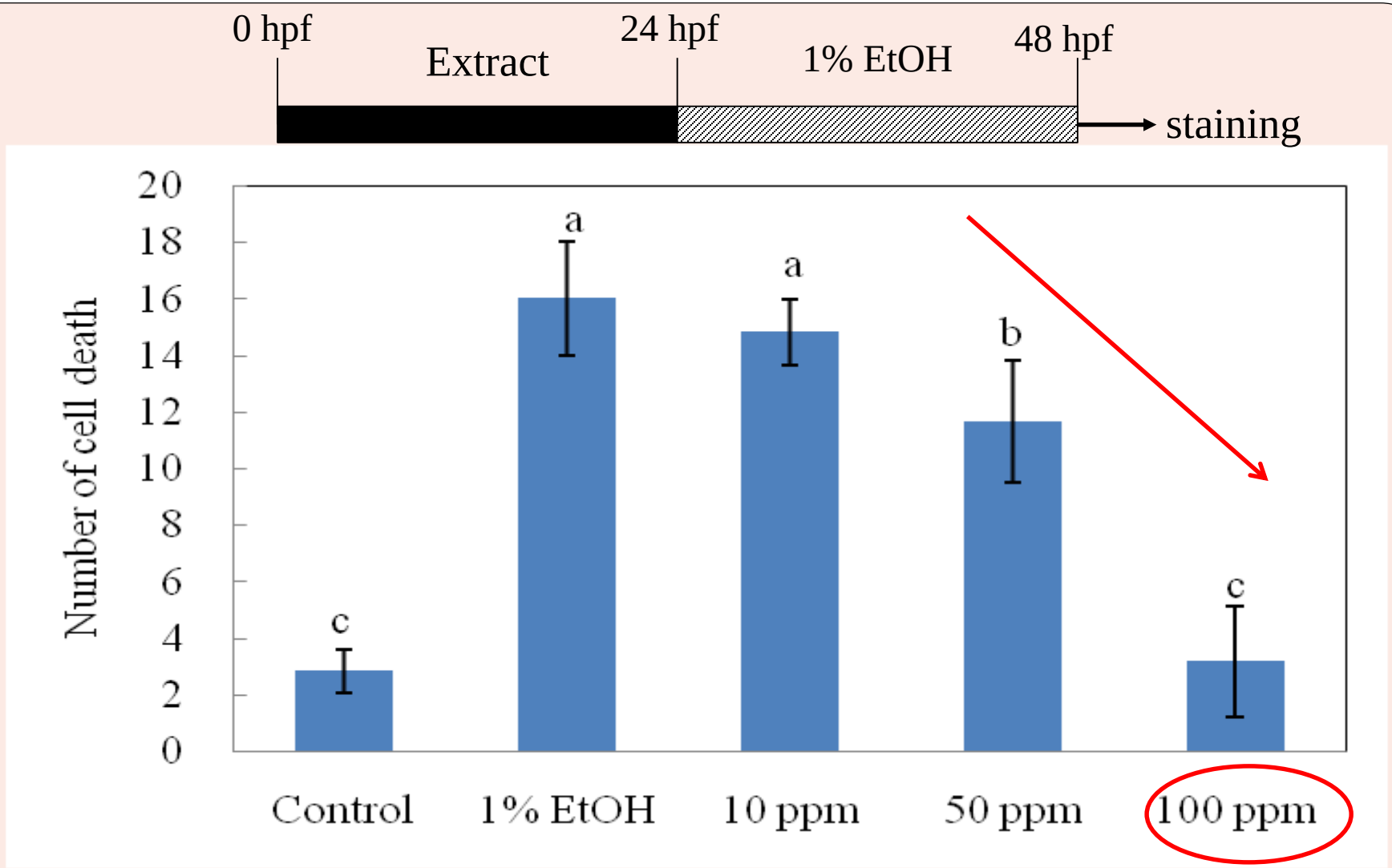


Fig. 4. Effect of protection with different concentration of PCEE on the number of died brain neuron cells in zebrafish embryo.

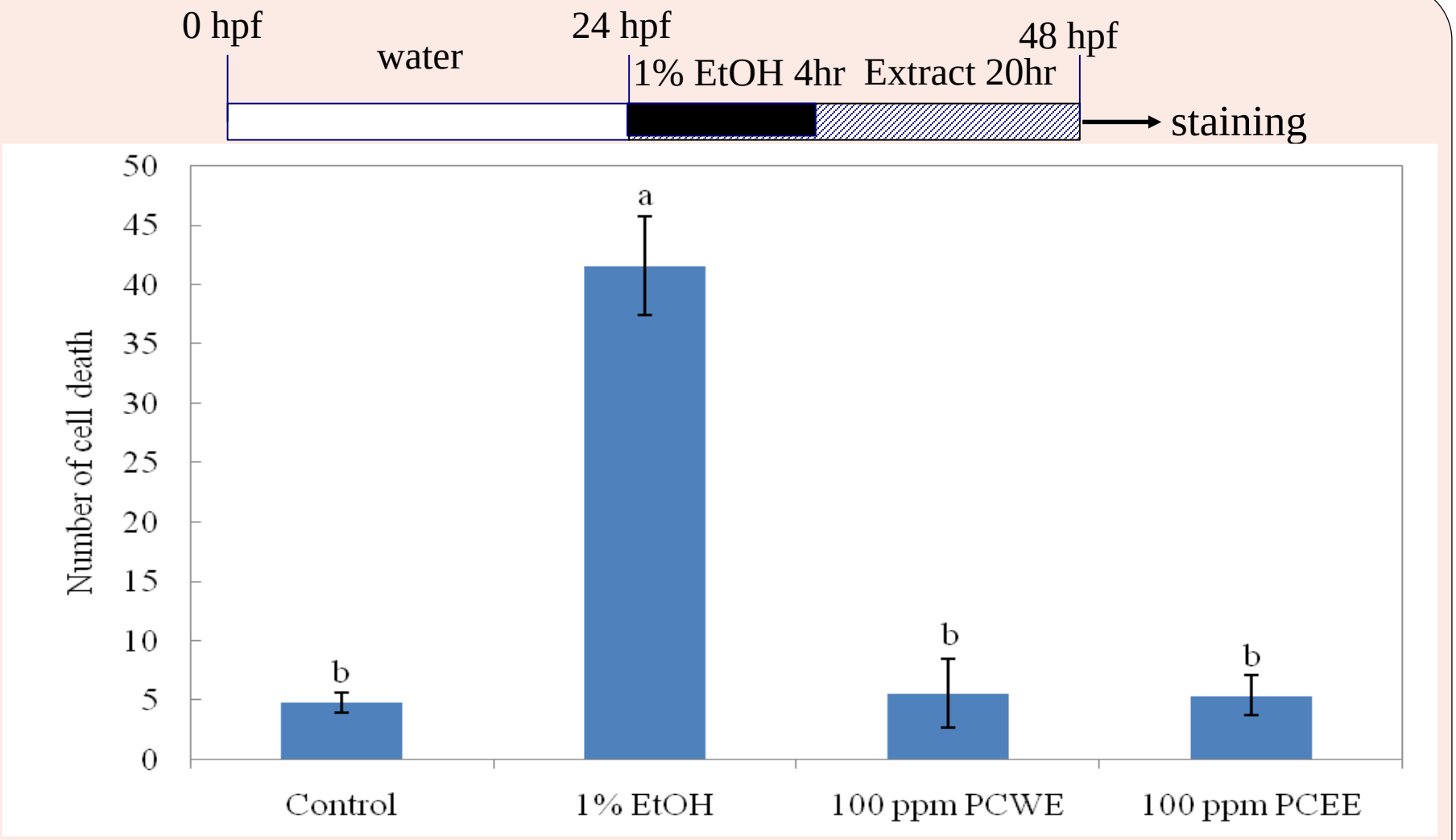


Fig. 5. Effect of repair with 100 ppm PCWE and PCEE on the number of died brain neuron cells in zebrafish embryo.

Conclusions

- After 2 weeks solid-state fermentation, both of the contents of crude polysaccharides and triterpenoids were highest in *Poria cocos* solid-state fermented soybean and brown rice mixture product.

- The 100 ppm water and ethanol extracts from *Poria cocos* solid-state fermented product had better protection and repair effects of brain cells in zebrafish embryos.

Thanks for your attentions!