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Research Article

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Antioxidant, Anticancer Cell Lines and Physiochemical Evaluation of Cobra Oil

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ABSTRACT

In this study, the oil was extracted from depot fat of Naja kaouthia, a cobra snake. The oil components were detected by GC-MS which showed 26.29% of saturated fatty acid, 31.42% of monounsaturated fatty acid and 23.74% of polyunsaturated fatty acid. The dominant components were Palmitic acid (17.47%), Vaccenic acid (22.98%) and Linoleic acid (16.80%). Biological functions of cobra oil were investigated for anti-oxidant and anti-cancer cells. Various concentrations of cobra oil (20%, 10%, 5%, 2.5% and 1.25%; v/v) could inhibit free radical scavenging by DPPH method 88.11%±1.62%, 54.84%±2.63 %, 35.10%±6.41%, 14.61%±2.37% and 6.01%±3.41% respectively. In addition, KATO-III, $HepG_2$ and SW620 cells undergo cytotoxic activity by cobra oil for 24, 48 and 72 h detected following MTT assay. As a great IC_{50} of cobra oil on KATO-III, $HepG_2$ and SW620 were 1.63% ±0.08% (at 72 h), 1.67% ±0. 22% (at 48 h) and 1.67% ±0.43% (at 48 h), respectively.

Keywords: *Anticancer, Antioxidant, Cobra oil, Fatty acid profile, Natural product*

INTRODUCTION

Natural oils are wildly traditional remedies for skin care and many diseases. The oil of plants and animals has been studied for pharmaceutical applications. Oil, extracted from many native crops, has been studied for pharmacological properties such as antimicrobial[1], anti-inflammatory[2,3,4], antioxidant activity[5], wound healing[6,7] and also anti-cancer[8]. The active components of natural oils were concerned with polyunsaturated fatty acids which affected diseases and cancers[8,9,10,11].

Snake oils have been used as traditional medicine for remedy to skin problems, local tissue necrosis and wound assessments. The famous snake oil, *Enhydris chinensis* (Chinese water snake), was a rich source of eicosapentaenoic acid (EPA; omega-3 PUFA) which had potential to muscle, anti-inflammatory and confers therapeutic benefits[12]. Fixed oil from *Boa constrictor* snakes were antimicrobial and anti-inflammatory activities[13].

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It had also shown 70% reducing actively proliferation on keloid fibroblasts of human skin[14]. In Thailand, cobra oil has been used as a broad traditional method of skin care. There is no detailed information about the composition and pharmacological properties of cobra oil. Scientific studies should be performed to better understand the value and safety of Thai herbal products. In this work, fatty acid components of the cobra oil were determined by GC-MS analysis. The cytotoxic activity of cobra oil was studied on three cancer cell lines (KATO-III, HepG2 and SW620). Cobra oil effects were investigated the anti-oxidant property by using DPPH method.

MATERIALS AND METHODS

Material and cell cultures

Human gastric carcinoma, KATO-III (ATCC®HTB-103), Human colon adenocarcinoma, SW620 (ATCC®CCL-227) and Human liver hepatoblastoma, $HepG_2$ (ATCC®HB-8065) were purchased from the American Type Culture Collection (ATCC, USA). All cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 1 mM glutamine, 100 U/ml streptomycin and penicillin and incubated at 37ºC with 5% CO_2. The stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Dichloromethane and MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide]) were purchased from Sigma (Sigma, St Louis, MO, USA). The other chemicals were analytical grade.

Antioxidant activity

Antioxidant activity was detected by DPPH free radical scavenging followed by Zhang et al. 2007[15]. Briefly, cobra oil was serial diluted 2 fold from 20% to 0.625% in dichloromethane (% v/v). Negative control (Dichloromethane only) was also tested in the same experiment. Two milliliter of each dilution was added with 2 ml of 0.16 mM DPPH solution and then mixed by vortex for 1 min. The mixtures were kept away from light for 30 min. DPPH color was changed from blue to yellow by anti-oxidant substance in cobra oil. An absorbance was measured at 515 nm by Elisa reader. Data were expressed as mean ± SD of four independent replicates. Equation of % DPPH inhibition was $[1-(A_{sample} - A_{blank})/A_{control}] \times 100$.

A_{sample} was OD_{515} of samples with DPPH.

A_{blank} was OD_{515} of samples without DPPH.

$A_{control}$ was OD_{515} of DPPH only.

Cytotoxic activity

Cytotoxic activity of cobra oil on Kato-III, $HepG_2$ and SW620 cells were determined by MTT assay. Briefly, the cells were seeded into 96 well plates (NUNC, Denmark) at concentrations of 1×10^5 cells/ml and incubated at 37ºC with 5% CO_2 for 24 h. The cells were treated with various concentrations of cobra oils (1.25, 2.5, 5, 10 and 20%, v/v) for 72 h. The oils were diluted in the complete RPMI1640 medium with 1% DMSO. MTT solution (2.5 mg/ml) was added and incubation continued for 3 h. Supernatant was carefully removed and added 100 µl DMSO to dissolve crystal formazan. Untreated cells were set as 100% of viability. Data were expressed as mean ± SE of three independent replicates.

Lipid extraction

The fat sack was immediately excised from the cobra (*Naja kaouthia*) snake newly death. Lipid was extracted from the fat sacks of cobra. Briefly, fat sacks were cut into small pieces and put into 20 ml barrel of a syringe and incubated at 37ºC until these cells became shrunken. The lipid was kept at -20ºC until further study.

Fatty acid profile analysis (FAMEs: Fatty acid methyl ester)

Two hundred microliters of lipid was simultaneously transesterified with 0.3 ml 5% HCl/MeOH and incubated at 85ºC for 1 hr. The sample was dissolved with hexane and detected by using a gas chromatography coupled to a mass spectrometer (GC/MS). The injector and detector were 250ºC split mode. One hundred microliters of sample was injected into an Innowax column (30m x 0.25 mm, film thickness 0.25 µm). The carrier gas was helium at a flow rate of 1.0 ml/min. The oven temperature was gradually increased from 50ºC to 200ºC at 20ºC/min and followed by 230ºC at 3ºC/min according to the function of instrument. The detector was recorded at 30 to 400 amu. The Wiley database was used as standard components for the spectrometer. Data were expressed as mean ± SE of three independent replicates.

RESULTS

Lipid extraction and lipid profile of cobra oil

Lipid components of cobra oil consisted of 26.29% saturated, 31.42% of monounsaturated and 23.74% of polyunsaturated fatty acids. The results represented 17.47% of Palmitic acid, 22.98% of Vaccenic acid, and 16.80% of Linoleic acid as the major components of saturated, monosaturated and polysaturated fatty acids, respectively (Table1).

Table1. Fatty acid components of cobra oil

Fatty acid components	%Quality	RT	%Area	± SE
Saturated fatty acids				
Undecylic acid	95	8.35	0.15	0.06
Myristic acid	97	9.60	0.71	0.18
Pentadecylic acid	81	10.34	0.18	0.06
Palmitic acid	99	11.23	17.47	3.54
Margaric acid	96	12.22	0.37	0.09
Stearic acid	99	13.43	4.81	0.30
Arachidic acid	92	16.31	0.25	0.02
Palmitinic acid	99	21.23	2.34	0.43
			26.29	
Monounsaturated				
Palmitoleic acid	99	11.49	4.01	1.48
Vaccenic acid	99	13.75	22.98	2.23
Oleic acid	99	13.81	3.84	0.39
Oleic acid†	91	16.66	0.59	0.09
			31.42	
Polyunsaturated				
Linoleic acid (LA)	99	14.37	16.80	2.60
Eicosatrienoic acid (ETE)	90	17.95	0.56	0.01
Eicosatetraenoic acid	89	18.37	0.68	0.14
Docosahexaenoic acid (DHA)	91	25.91	4.45	0.56
			23.74	
Cholesterol, Pheromone etc.			-	
		Total	81.45	

Scavenging activities of Cobra oil

Cobra oil was found to have significant % DPPH inhibition as compared to negative control. The percent of DPPH inhibition depended on the dosage of cobra oil (Figure1). Twenty and 1.25% were the highest and the lowest of cobra oil concentrations which exhibited 88.11% ± 1.61% and 6.01% ± 3.41% of DPPH inhibition, respectively.

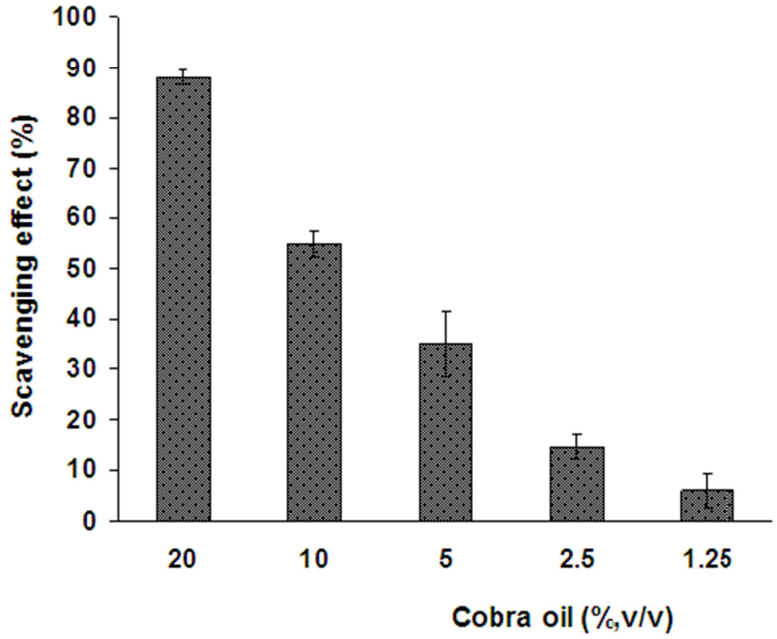

Fig.1: Free radical scavenging activities (%) of cobra oil

Cytotoxic activity

The cytotoxicity effect of cobra oil on cancer cell line was investigated by MTT assay. Three cancer cells; KATO-III, $HepG_2$, SW620 were treated with various concentrations for 24, 48 and 72 h of incubation. Inhibition concentration (IC_{50}) of cobra oil on KATO-III was 3.16% ± 0.47%, 2.14% ± 0.35% and 1.63% ± 0.08%, respectively. In this cell, IC_{50} was reduced by time of incubation. The IC_{50} of $HepG_2$ cell was 2.79% ± 0.58%, 1.67% ± 0.22% and 1.69% ± 0.27% while the IC_{50} of SW620 was 2.50% ± 0.59%, 1.67% ± 0.43% and 1.75% ± 0.26%, respectively. Cobra oil had highest effectiveness on $HepG_2$ and SW620 cells at 48 h of incubation. There were not changed at long incubation periods (Figure2).

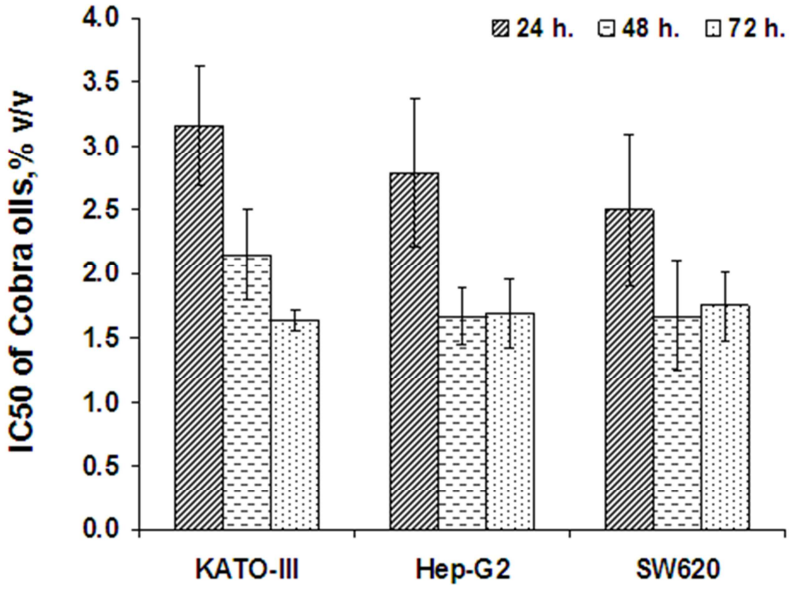

Fig. 2: The IC_{50} of cobra oil on three cancer cell lines

DISCUSSION

The depot fat of snake was cut and blended, then incubated at 37ºC until lipid came out. This method was easy, no extra instruments and no specialist were needed. The oil was extracted by the simple method and the results were similar to those using the Soxhlet apparatus[16]. Unsaturated fatty acids (55.16%) were found in higher amounts than saturated fatty acids (26.29%) in cobra oil. There was not a clear correlation between unsaturated fatty acids and saturated fatty acids of cobra oil to its biological functions. Palmitic acid (17.47%) was the dominant saturated fatty acid of cobra oil which is also found in various reptile oils[16,17,18]. While, Vaccenic acid (22.98%) and Linoleic acid (16.80%) were monounsaturated and polyunsaturated fatty acids.

The biological functions of snake oils had been rarely reported. This is the first report to scavenge free DPPH radical property of cobra oil derived from fat sacks of *Naja kaouthia*. Our study reveals that cobra oil is an abundant of fatty acids which as an effective natural antioxidant. Cobra oil has potent to scavenge free DPPH radicals which are similar to the effect of the oils of other animals and plants[19,20,21]. A lot of polyunsaturated fatty acids which found in the cobra oil may be act as antioxidant. However, the antioxidant effect of cobra oil was not clear. It might be an action of unsaturated fatty acid contents or by the effect of the other chemical components in the oil[22] or occurred by the experimental condition[23].

In addition, cobra oil had cytotoxic activity on three cancer cells; KATO-III, $HepG_2$ and SW620. Cell counts in KATO-III consistently decreased between 24 h to 72 h while cell counts of $HepG_2$ and SW620 declined between 24 h and 48 h of incubation. As previously reported, *Boa constrictor oil* (BCO) was strongly on proliferation inhibition of keloid and normal dermal fibroblasts cells more so than shea butter *in vitro*[24]. An active gradient in BCO was referred to omega-3 polyunsaturated fatty acid especially eicosapentaenoic acid (EPA) which was not found in cobra oil. Therefore, some components in cobra oil could possibly have cytotoxic effect on three cancer cells. The isolation of fatty acid should be considered further.

CONCLUSION

Cobra oil was abundant of fatty acids which used to remedy of healthcare without scientific-base study. This was an initial study of Thai traditional herbal, cobra oil. The oil was extracted by a simple method which might easily be provided to people in remote areas of Thailand. The oil has the potential to inhibit the proliferation of cancer cells and potent antioxidant activity. However, mechanisms of action of cobra oil should be studied further.

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