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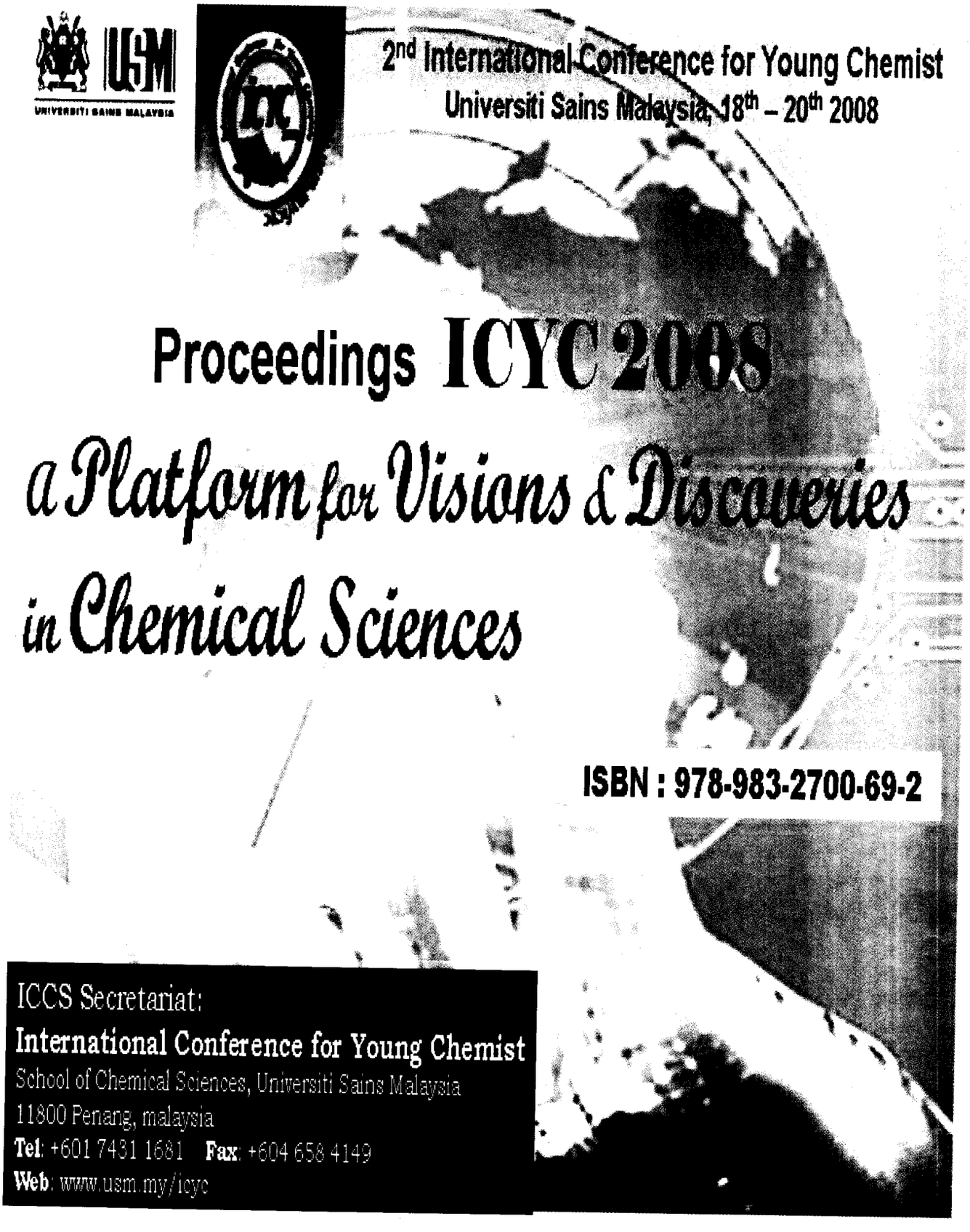

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CHEM/I-5

ACUTE AND SUBCRONIC TOXICITY OF ETHANOLIC EXSTRACT FROM STEM BARK OF *HOPEA MENGARAWAN*

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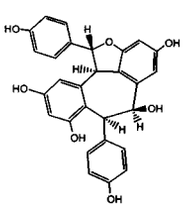
ABSTRACT

This research was intended to investigate the acute and subcronic toxicity of ethanolic extract from stem bark of Hopea mengarawan. Acute toxicity investigation on mice male Swiss revealed that LD_{50} of this extract on mice was 2.290 mg/ kg body weight, hence categorized as 'litle-toxic substance'. whereas sub cronic toxicity is conducted with to gift extract at white mouse by per oral for 3 month continuously. In range of time referred will be perceived body weight and consumption eat every week, behavior appearance and all abnormalities, laboratory test of histopatologis organ in mouse, SGOT, and SGPT, and observed hystologic in microscopic do well by mouse that has been healthy or died after experiment ends.

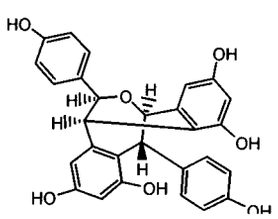
***Key words**: Toxicity; Hopea mengarawan*

INTRODUCTION

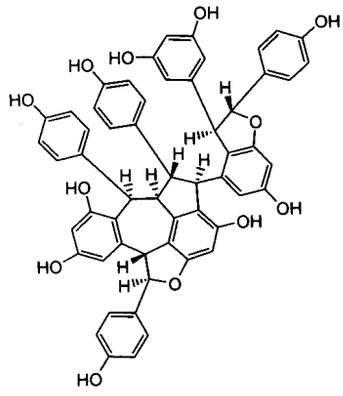
Antihepatotoxic activity tes *in vivo* of ethanolic extract of stem bark of *H. odorata. H. mengarawan* and *H. nigra* can reduce concentration of SGPT (Serum Glutamat Piruvat Transaminase) of rats, that induction by CCl_4 and also disappepar of necrocis of the lever (Sri Atun, dkk, 2005[a,b]; 2006[a,b]). In this research can be known four compound isolated from *H. mengarawan* have antihepatotoxic actvity, that are balanocarpol (**1**), heimiol A (**2**), vaticanol B (**3**), and vaticanol G (**4**).



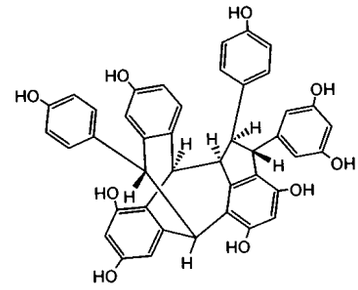
Balanocarpol (**1**)



Heimiol A (**2**)



Vaticanol B (**3**)



Vaticanol G (**4**)

To develop the extract of stem bark of plant *Hopea*, specially *H. mengarawan* become the product phytopharmaca which can be used as a new drug of antihepatotoxic which standardize, safe, with quality, require to be conducted] by toxicity test, acute toxicity, subcronic toxicity at lever and kidney, and also teratogenic test. Result of this test will represent the especial consideration to its use as traditional drug and to clinical test of human being at phase research hereinafter. In this article will be studied by result of acute and subcronic toxicity tests of ethanol extract *H. mengarawan*.

EXPERIMENTAL

Instruments : glass ware, micropippette, vacum evaporator, analitical balance

Animal tests : male mice Swiss strain, age 2 months, body weight 30-32 g, white mouse type male whistar age of 2-3 month body weight 300 g.

Materials : ethanolic extract of stem bark of *H. Mengarawan* (from garden of attempt of Dramaga and Jasinga, Bogor at June 2007; the stem bark dried and grind), formalin, CMC-Na, ethanol, and hematoxilin

Extraction. Process of extraction by maseration of stem bark powder of *H. Mengarawan* which age have more than 40 years by using ethanol at room temperature, during 24 hours. The maseration repeated by as much 2-3 times. Extract obtained to be collected and condensed by vacuum evaporator.

The acute toxicity test: The acute toxicity test conducted by using male mice of Swiss strain with the age more or less 2 months, healthy and own the body weight more or less of equal (30-32 g).The mice used in this research is adapted environmentally at the research place (laboratory of medical faculty of Gadjah Mada University). To determine the variation of dose require to be conducted by orientation test to know highest dose is which technically admit of passed to a animal test, while lowest dose is minimum dose which can generate effect. Dose got from orientation used as a highest dose, after that searched by the its fold factor and determined its variation dose to each group. The variation Dose determined to use the following formula :

$$F = \sqrt[r]{\text{highest / lowest dose}}$$

r = sum of variation of concentration -1
F = fold factor

In this research, the extract gift to mice by peroral and selected by three dose, (the dose killing less than 10%, 50%,and more than 90%). The observation conducted by during 14 days, covering autonomic, behavioral, sensoric, neuromuscular, cardiovasculer, inhalation, eye, gastrointestinal, and skin. And so do will be conducted histopatologi microscopicly do well by the mice which still be healthy or die after the end of experiment.

The subcronic toxicity test : The subcronic toxicity test of ethanol extract *H. mengarawan* are conducted to white mouse type male whistar age of 2-3 month, body weight around 300 g. The sub chronic toxicity test conducted to determine characteristic and effect place toxic, beside determine level dose of without effect. In this research selected three doses, namely high enough dose to generate sign toxicity, but insufficient high to kill a large part of that animals, low dose expected not give toxic effect, and middle dose. Observing times during 3 month. In range of time referred will be observed body weight and consumption eat every week, behavior appearance and all abnormalities, laboratory test observed of histopatologis organ in mouse, clinic test covers glucose of fasting blood, SGOT (Serum Glutamat Oksaloasetat Transaminase), SGPT (Serum Glutamat

Piruvat Transaminase) do well by mouse that has been healthy or died after experiment ends.

RESULT OF RESEARCH AND DISCUSSION

To determine the dose to be used in the toxicity acut test conducted by by giving extract ethanol *H. mengarawan* certain in solvent CMC-NA 1%, and observation the amount of mice that death like there are at table 1.

Dose got from the orientation result knowable highest dose is 5,337mg/ bw, lowest dose is 1,670mg/ bw. From the data is used to look for the its fold factor and its variation dose to each group. The variation dose determined to use the the following formula above:

From calculation result obtained, F = 1.473, so that variation of dose used in acut toxicity test are 1,670; 2,460; 3,623; and 5,337mg / kg bw. Observation conducted by during 14 days, covering autonomic, behavioral, sensoric, neuromuscular, cardiovasculer, inhalation, eye, gastrointestinal, and skin. And so do will be conducted histopatologi microscopic do well by the mice which still be healthy or die after the end of experiment. Data of death Perception after 24 clock until 14 days of there are in table 2.

From calculated by above tables of LD_{50} with the linear equation regresi (logarithm of dose of vs probit), the equation of regression is, Y = 7.594 X - 20.516, at the value of r = 0.974. From the equation obtained by value of LD_{50} 2.290 mg/ kg bw. The value of LD_{50} indicate that the extract of ethanol of stem bark powder of *H. mengarawan* have the character of a few/little toxic (Loomis, 1978). Observation to mice conducted after extract gift until 14 day, covering body weight, autonomic, behavior, sensoric, neuromusculer, cardiovasculer, inhalation, eye, gastrointestinal, and skin. The histopatology microscopic do well by the mouse which still be healthy or die at the end of this experiment. Observation Histopatology conducted to all organ in mice covering heart, liver, right and left kidney, stomach, and spleen. Observation in physical, behavior, and body weight each mice showed the difference inexistence which significant between control and experiment group, while observation by histopatologis of organ in mice resulted data at table 3. The histopatologis data showed small difference condition at liver, lungs, and spleen but not significant between control and experiment group.

Result of subcronic toxicity test. Measuring of enzyme activity SGOT and SGPT from animal serum showed at table 4 and table 5. Analysis one way anava from enzyme activity SGOT gives F-calculate result lower than F tables (□=0,01) we concluded that not existed difference has a meaning between control group and all treatment groups. And so from analysis enzyme SGPT activity with one way anava gives result F calculate lower than F tables (□= 0,01) so we concluded that not existed difference has a meaning of enzyme activity GPT between control group and all groups experiment. This result indicated that enzyme activity SGOT animal tries after treatment has stayed in span of normal nevertheless for enzyme activity SGPT is existed increase nevertheless not have a meaning . This Result showed that not side effect hepatotoxic from this ethanol extract *H. mengarawan*. Toxicity to function faal *ren* is specified with analysis urine by measurement concentration of leucosit, urobilinogen, protein, glucose, keton, bilirubin, pH, and sediment from animal control and experiment with result at tables 6. From this result can be concluded that test extract not showed effect toxic to animal kidney at subcronic usage. Observation Histopatology conducted to all organ in mice covering *heart, hepar, gastrium, pulmo, lien*, and ren. The histopatologis (Table 7) data showed small difference condition at this organ but not significant between control and experiment group.

Table 1. Data of determined dose

Group	Dose	Amount of mice	Amount of the death mice	% of death
1	1,670 mg/kg bw	4	0	0 %
2	2,460 mg/ kg bw	4	1	25 %
3	3,623 mg/ kg bw	4	2	50 %
4	5,337 mg/ kg bw	4	4	100 %
5	7,862 mg/ kg bw	4	4	100 %

Table 2. Data of the death mice in acute toxicity tests

Group	Dose	Amount of mice	Amount of death	% of death	probit
control	0,75 ml CMC-Na	6	0	0%	-
1	1,670 mg/kg bw	6	1	16 %	4.01
2	2,460 mg/ kg bw	6	4	66.67 %	5.43
3	3,623 mg/ kg bw	6	5	83.33 %	5.96
4	5,337 mg/ kg bw	6	6	100 %	8.09

Table 3. Data of histopatologis of organ in mice after experiment

Group	Code	liver	Ren	lungs	spleen	Cardiovascular	Stommach-esoph
Control	K -24 hours	-	-	PI	-	-	-
	K-14 day	MFN	-	PI	-	-	-
D-1	O -24 hours	MFN,DM	-	PI	-	-	PK and N
	O-14 day	-	-	PI	N	-	-
D-2	I-24 hours	-	-	PI	N	-	-
	I-14 day	MFN	-	O	-	-	-
D-3	II-24 hours	-	-	O	MK	-	-
	II-14 day	MFN,DM	-	PI	-	-	-
D-4	III-24 hours	-	-	O	MK	-	-
	III-14 day	MFN, DM	-	PI	-	-	-

Note :
MFN = multi fokal necrosis
DM = vakuola citoplasma on hepar cell
PI = Pulmo interalveolaris
O = acumulation of homogen eosinofilik at lumen alveoli
N = Necrosis at pulpa
K = incresed of megakariosit at limpa
PK and N = Proliferasi kelenjar and necrosis
D1-D4 = dose 1-4

CONCLUSION

The acute toxicity test of extract ethanol of stem bark *H. mengarawan* conducted to male mouse of Swiss strain obtained by value of LD_{50} 2,290mg/ kg bw, having the character of a little toxic, so that the extract peaceful relative if used as by drug at certain dose. Despite that the extract not showed effect subcronic toxicity to liver function and kidney function mouse up to highest dose 300 mg/ kg bw within tests 3 month with per oral usage at once a day.

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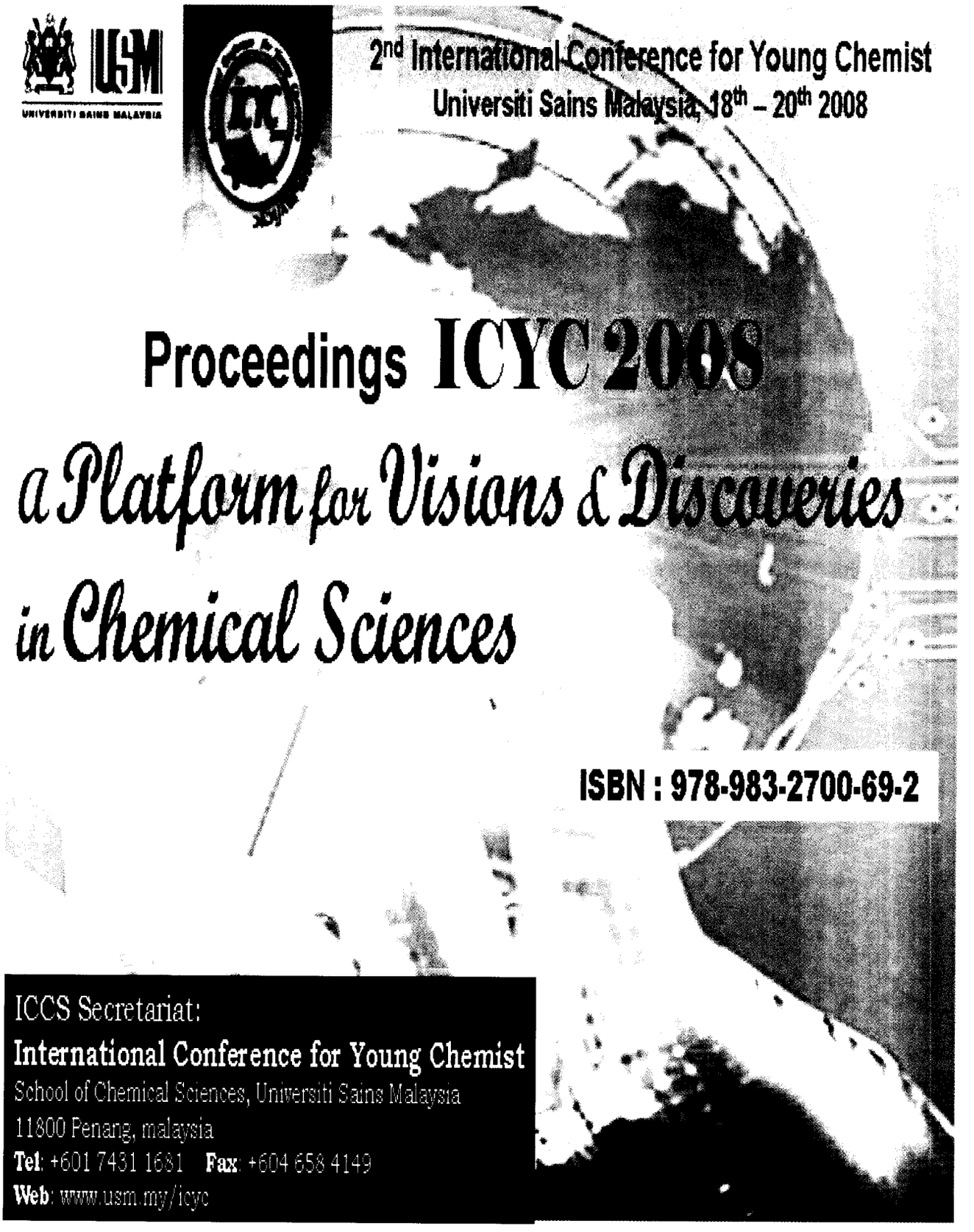

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