Đánh giá hoạt tính chống khối u của Saphia Alkali K90 trên mô hình gây ung thư bằng DMBA ở chuột Swiss

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14. Evaluation of antitumor activity of saphia alkali K90 on dmba-induced carcinogenesis in swiss mice

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Tóm tắt

The present study aimed to initially evaluate the anti-tumor effect of orally administered Saphia Alkali K90 in 7,12-dimethylbenz(a)anthracene (DMBA)-induced carcinogenesis in Swiss mice. Mice exposed to DMBA (total dose of 6 mg, p.o.) were treated with SAK90 at 15 ml/kg BW and 45 ml/kg BW daily. The vehicle was given to both normal and diseased controls. The change in body weight, mortality rate, tumor incidence, tumor burden, tumor yield, histopathology, and hematological and biochemical parameters were evaluated after 16 weeks of treatment. 23,26% of DMBA-exposed mice presented neoplasms. SAK90 tended to reduce death, tumor incidence, and tumor yield compared to the DMBA group. The formulation exhibited protective effects against DMBA-induced hepatotoxicity through a trend in drop transaminase levels and increased albumin concentration concurrently compared to the DMBA group. This study suggested that SAK90 has potential anticancer activity.

Từ khóa

Saphia Alkali K90, tumor, DMBA, mice

Tài liệu tham khảo

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EVALUATION OF ANTITUMOR ACTIVITY OF SAPHIA ALKALI K90 ON DMBA-INDUCED CARCINOGENESIS IN *SWISS* MICE

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The present study aimed to initially evaluate the anti-tumor effect of orally administered Saphia Alkali K90 in 7,12-dimethylbenz(a)anthracene (DMBA)-induced carcinogenesis in Swiss mice. Mice exposed to DMBA (total dose of 6 mg, p.o.) were treated with SAK90 at 15 ml/kg BW and 45 ml/kg BW daily. The vehicle was given to both normal and diseased controls. The change in body weight, mortality rate, tumor incidence, tumor burden, tumor yield, histopathology, and hematological and biochemical parameters were evaluated after 16 weeks of treatment. 23,26% of DMBA-exposed mice presented neoplasms. SAK90 tended to reduce death, tumor incidence, and tumor yield compared to the DMBA group. The formulation exhibited protective effects against DMBA-induced hepatotoxicity through a trend in drop transaminase levels and increased albumin concentration concurrently compared to the DMBA group. This study suggested that SAK90 has potential anticancer activity.

Keywords: Saphia Alkali K90, tumor, DMBA, mice.

I. INTRODUCTION

Cancer, also known as neoplasm or malignant tumor, is a large group of diseases that can begin in almost any organ or tissue of the body when abnormal cells grow uncontrollably, exceeding their normal boundaries to invade adjacent parts of the body and/or spread to other organs.[1] Cancer is the second leading cause of death globally, with an appraisal of 9.9 million deaths in 2020, and is predicted to continue to increase, with an estimated 30,2 million new cases per year by 2040.[2] Despite all the great stalks that have been made in cancer treatment over the past 50 years, it continues to be a significant health problem, so extensive efforts have been devoted to discover new treatment methods. One of such approaches is to explore natural plant products with anti-cancer properties.

Since traditional herbal medicines, such as extracts from herbal mixtures, frequently exhibit anticancer activities with negligible or no side effect, their use has been reexamined.[3] Numerous studies have shown that herbal medicine can promote apoptosis of tumor cells while blocking their growth and metastasis, including those of the lung, breast, colorectal, and other tumor types.[4] Combining extracts from herbal mixtures with antitumor medications in cocktail therapies may have synergistic effects against tumor growth, enabling lower doses of anticancer drugs. Therefore, it would be of great interest to combine the use of an anticancer medication and an herbal mixture to increase the positive effects of cancer chemotherapy.

Functional food Saphia Alkali K90 (abbreviated as SAK90), a multiplant production, was developed by Kiem Saphia Joint Stock Company. SAK90 composed of several constituents - *Hedyotis diffusa*, *Scutellaria barbata*, *Perilla frutescens*, and *Wedelia chinensis*- has demonstrated anti-

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cancer properties and effective results in treating certain types of cancer with recent advances in pharmacological practice.[5] The biological activities of important secondary metabolites are largely responsible for these medicinal herbs' antitumor effects.[5]

The present study was designed to initially examine the anticancer efficacy of polyherbal formulation SAK90 in *Swiss* mice bearing 7,12-dimethylbenz[a]anthracene (DMBA) induced cancer. DMBA is a prototype polycyclic aromatic hydrocarbon that has been used to induce tumors in animals. Tumors caused by DMBA exposure in mice match the multistep process occurring in humans and are heterogeneous, hence this model has been widely utilized to research cancer progression and prospective treatment approaches.[6,7]

II. MATERIALS AND METHODS

1. Saphia Alkali K90 product

Saphia Alkali K90 product (abbreviated as SAK90) was provided by Kiem Saphia Joint Stock Company. Each 100 mL of the product contains *Celastrus hindsii* (3.0 g); *Pseuderanthemun palatiferum* (2.5 g); *Hedyotis diffusa* (2.0 g); *Scutellaria barbata* (2.0 g); *Phyllanthus urinaria* (2.0 g); *Eclipta prostrata* (2.0 g); Guava (*Psidium guajava* L.) Leaves (2.0 g); *Perilla frutescens* (2.0 g); *Solanum procumbens* (2 g); *Mentha arvensis* (1.5 g); *Wedelia chinensis* (1.5 g); *Lactuca indica* (1.5 g); *Ilex kaushue* (1.5 g); *Amaranthus spinosus* (1.5 g); *Achyranthes aspera* (1.5 g); *Adenosma caeruleum* (1.5 g); *Crinum latifolium* (1.5 g); Microelements are activated from rare earths. Additional ingredients: Pure water enough for 100 mL.

The estimated human dose of SAK90 was 60-75 mL/day divided into three doses. SAK90 was diluted in distilled water and administered to the mice by oral gavage at 15 mL (low dose) and 45 mL (high dose) per kg b.w/day, based on the conversion (dose extrapolation factor of 12) from an equivalent dose of 75 mL/day and 225 mL/day for patients weighing approximately 60 kg in the clinic, respectively. The doses were prepared fresh daily and taken one hour before meals to optimize absorption.

2. Acclimatization of animals

A total of 80 adult *Swiss* mice (weight range, 24–34 g) of both genders (National Institute of Hygiene and Epidemiology, Vietnam) were used for the experiment. The animals were maintained in an air-conditioned room at a temperature of 22±1^{0}C and a humidity of 55±1% with a 12-hour light/dark cycle. They were fed a standard commercial rodent pellet diet (National Institute of Hygiene and Epidemiology) and had ad libitum access to water. The animals were allowed to acclimate to the environment for five days before the experimental session at the Department of Pharmacology, Hanoi Medical University. All animal procedures were performed under the ethical considerations regarding animal experimentation.

3. Carcinogenesis model

Carcinogenesis was induced by 7,12-dimethylbenzanthracene (DMBA) (Sigma-Aldrich), dissolved in olive oil, and administered by gastric gavage needle. Each animal received 1 mg per week until completing the total dose of 6 mg.[7] DMBA induction was performed in all groups except the healthy group, which received an equivalent volume of olive oil.

4. Experimental design

Animals were divided randomly into 4 groups of 20 animals each:

- Group 1 (Normal control): Mice were administered distilled water only.

- Group 2 (DMBA control): DMBA-treated

animals without additional treatments.

- Group 3 (SAK90 45 mL/kg): DMBA-treated animals supplemented orally with 45 mL/kg of body weight of SAK90 daily for 16 weeks.

- Group 4 (SAK90 15 mL/kg): DMBA-treated animals supplemented orally with 15 mL/kg of body weight of SAK90 daily for 16 weeks.

All animals were sacrificed at the end of the 16-week treatment period. Blood samples were collected through the carotid arteries for analyzing various hematological and biochemical parameters. All macroscopic tumors were counted, excised, and kept in formalin solution (10%) until the completion of the slaughter and the beginning of analysis of histological examination.

The following parameters were taken into consideration for the study:

Body weight: The body weight of the experimental mice was measured periodically during the experimental tenure.

Tumor parameters:

Tumor number: the overall number of tumors developed in each group;

Tumor incidence: the number of mice with tumors/survival animals expressed as a percentage incidence;

Tumor burden: the average number of tumors per malignant tumor-bearing mouse;

Tumor yield: the average number of tumors per survival mouse;

Hematological parameters: Blood collection from carotid arteries was carried out and the samples (0.3 ml) in EDTA were used for the assessment of hematological parameters such as hemoglobin (Hb), hematocrit, red blood cell (RBC), total white blood cells (WBCs), platelets by hematology analyzer (ABX Micros ES 60, Horiba Medical, France).

Biochemical parameters: Blood collected in non-heparinized tubes was then centrifuged at 3000 r/min for 10 min. The separated serum was analyzed by the Clinical chemistry analyzer Erba Chem 5v3 (India), for the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), albumin (ALB), total bilirubin (TBIL), and creatinine (CREA).

Histopathological examination: Tumor tissue specimens were fixed into 10% formalin for 24 h and processed by a conventional method, embedded in paraffin, sectioned at 4–5 μm and stained by hematoxylin and eosin for histopathological investigation under light microscope.

5. Statistical analysis

The data were processed in Microsoft Excel and analyzed using IBM SPSS Statistics software. The results were expressed as the Mean ± Standard Deviation (SD) and presented in tables and graphs. Statistical differences between the groups were determined using Fisher's exact test, Student's t-test, and paired sample t-test. P-values less than 0.05 are considered to be significant.

III. RESULTS

1. Effect of Saphia Alkali K90 on body weight

Table 1. Effect of Saphia Alkali K90 on body weight

Groups	Body weight (gram)				
	Before treatment	4th week	8th week	12th week	16th week
Normal control	29.1 ± 7.1	37.3 ± 6.1***	39.5 ± 7.2***	41.3 ± 8.4***	46.1 ± 7.9***
DMBA control	29.0 ± 3.8	35.9 ± 5.3***	37.9 ± 5.2***	41.3 ± 6.2***	45.5 ± 9.0***
SAK90 45 mL/kg	28.9 ± 5.1	34.5 ± 3.5***	36.8 ± 3.8***	40.6 ± 4.5***	43.3 ± 6.2***
SAK90 15 mL/kg	29.0 ± 4.4	35.4 ± 4.9***	37.4 ± 6.0***	40.9 ± 6.2***	44.3 ± 7.6***

****p<0.001 as compared to before treatment (paired samples t-test).*

Table 1 displays the test product's impact on variations in body weight. Positive changes in body weight were seen in all the groups. There was no discernible difference in body weight between the normal control and DMBA-exposed (Group 2, 3, and 4) groups.

2. Effect of Saphia Alkali K90 on mortality rate

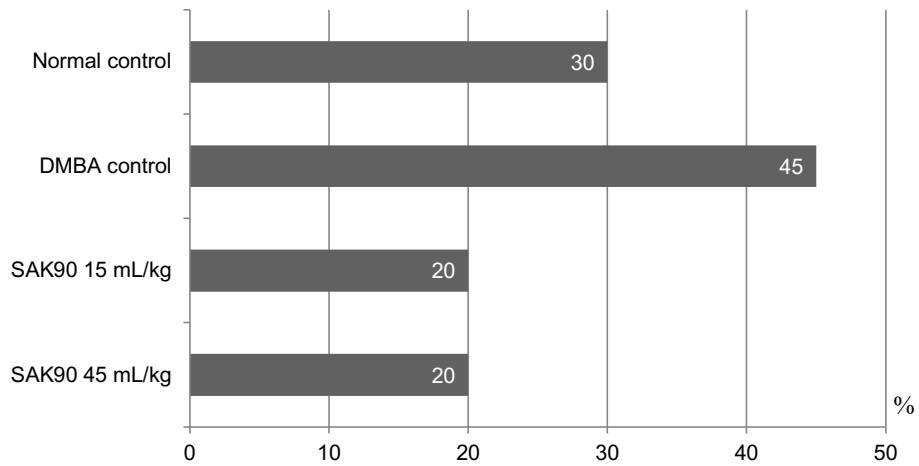

Figure 1. Effect of SAK90 on the mortality rate of DMBA-treated mice

Figure 1 depicts the effects of SAK90 on the mortality rate of mice given DMBA. The DMBA control group had the highest mortality rate, 1.5 times that of the normal control mice and 2.25 times that of both SAK90 groups; nevertheless, the difference was not statistically significant.

3. Effects of SAK90 on hematological and biochemical parameters

After 16 weeks of treatment, the effects of SAK90 on hematological and biochemical parameters were assessed (Table 2). In the DMBA control group, the number of white blood cells tended to increase more than in the normal control group. The white cell count was considerably lower in the SAK90-treated groups compared to the DMBA control group ($p < 0.05$). When all study groups were compared, there was no difference in red cell and platelet indices ($p > 0.05$). When all study groups were examined, there were also no

difference in serum levels of transaminases (AST, ALT), total cholesterol (TC), total bilirubin (TBIL), and creatinine (CREA) ($p > 0.05$). The serum albumin concentration significantly decreased in the DMBA-administered groups compared to the normal control group that only received the DMBA diluent, olive oil ($p < 0.01$ or < 0.05). Although the difference was not statistically significant ($p > 0.05$), serum albumin concentration tended to increase higher in the SAK90 groups than in the DMBA control group.

Table 2. Effects of SAK90 on hematological and biochemical parameters

Parameters	Normal control	DMBA control	SAK90 45 mL/kg	SAK90 15 mL/kg
WBC $10^3/mm^3$	8.0 ± 1.8	9.5 ± 2.5	7.4 ± 2.2#	7.1 ± 2.2#
RBC $10^6/mm^3$	12.1 ± 1.2	12.3 ± 1.5	12.0 ± 2.0	12.2 ± 1.1
HGB g/dL	12.8 ± 1.2	12.7 ± 1.5	11.7 ± 1.6	12.4 ± 0.8
HCT %	56.1 ± 5.5	56.3 ± 8.5	53.3 ± 8.5	57.0 ± 4.1
MCV μm^3	46.8 ± 2.2	45.8 ± 3.7	45.1 ± 3.3	46.9 ± 2.6
PLT $10^3/mm^3$	945.9 ± 208.2	985.0 ± 186.5	995.9 ± 222.3	867.6 ± 111.2
AST UI/L	178.9 ± 52.6	212.7 ± 81.2	206.1 ± 92.3	172.7 ± 54.2
ALT UI/L	55.8 ± 16.0	70.5 ± 22.9	57.1 ± 19.8	55.6 ± 19.7
TC mg/dL	86.4 ± 18.4	78.6 ± 19.7	89.7 ± 16.7	82.9 ± 16.1
ALB mmol/L	3.40 ± 0.12	3.12 ± 0.28**	3.25 ± 0.21*	3.21 ± 0.22*
TBIL mmol/L	8.7 ± 0.3	8.7 ± 0.3	8.6 ± 0.3	8.7 ± 0.3
CREA µmol/L	42.9 ± 2.8	44.8 ± 3.3	42.4 ± 3.5	44.6 ± 3.2

#p<0.05 as compared with DMBA control animals (Student's t-test)

**p<0.05; **p<0.01 as compared with normal control animals (Student's t-test)*

4. Effects of Saphia Alkali K90 on tumor parameters

Table 3. Effect of Saphia Alkali K90 on tumor incidence

Groups	Tumor incidence			
	Gross appearance		Microscopic examination	
DMBA control	7/11	(63.6%)	4/11	(36.4%)
SAK90 45 mL/kg	9/16	(56.3%)	4/16	(25.0%)
SAK90 15 mL/kg	5/16	(31.3%)	2/16	(12.5%)

Observing the data in Table 3, the low-dose SAK90 group had the lowest incidence of tumors based on gross observation (the number of mice with suspected tumors) and microscopic evaluation (the number of mice with tumors verified by histopathology). However, the difference is not statistically significant when compared to the DMBA control group ($p > 0.05$).

Table 4. Effects of SAK90 on tumor number, tumor burden, and tumor yield

Groups	Tumor number		Tumor yield	Tumor burden
	Gross appearance	Malignant on histopathology		
DMBA control	09	05	5/11 (0.45)	5/4 (1.25)
SAK90 45 mL/kg	13	06	6/16 (0.38)	6/4 (1.50)
SAK90 15 mL/kg	08	03	3/16 (0.19)	3/2 (1.50)

Mice belonging to Groups 3 and 4 with SAK90 administration revealed a comparative reduction in tumor yield to 0.38 and 0.19 (the DMBA control value being 0.45), respectively (Table 4). There was no improvement in tumor burden when comparing the SAK90-treated and DMBA control groups.

The histopathologically confirmed malignant tumors that occurred in DMBA-exposed mice are listed in Table 5.

Table 5. The histopathologically confirmed malignant tumors

Groups	Histomorphology
DMBA control group	01 Squamous cell carcinoma
	02 Lung cancer
	01 Renal cell carcinoma
	01 Hepatocellular carcinoma
High-dose SAK90	01 Keratinizing squamous cell carcinoma
	04 Non-Hodgkin's lymphoma (subcutaneous tissue, kidney, mesenteric mass)
	01 Hepatocellular carcinoma
Low-dose SAK90	02 Non-Hodgkin's lymphoma (kidney)
	01 Keratinized esophageal squamous cell carcinoma

III. DISCUSSION

The central cause of the rising pollution is modernization and the growing need to keep up with it. The global production of genotoxic polycyclic aromatic hydrocarbons (PAHs) is a result of rising pollution. In experimental cancer research, DMBA, a synthetic PAH, has long been used as a mutation-inducing agent in cancers of the breast, lung, lymphoid tissue, intestine, and skin.[7] DMBA is changed into DMBA-3,4-diol-1,2-epoxide (DMBA-DE) by cytochrome p450 enzymes through a sequence of reactions. The ultimate carcinogen then interacts with DNA to form adducts that are accountable for mutations and cancer.[8] In the present study, mice received 1 mg/week of DMBA diluted in olive oil orally for the first six

weeks of the study to induce carcinogenesis. Previous research found that tumors appeared in the tenth week after the initial treatment of DMBA. However, the vast majority of tumors emerged in the 16th week.[6,7] Therefore, SAK90 will be simultaneously administered daily for 16 consecutive weeks to evaluate the effect of shielding genetic material from DMBA-induced damage, thereby preventing tumor growth in study animals.

An essential measure for researching the harmful effects of chemicals is body weight. Table 1 displays data indicating an increase in the animals' body weight in both SAK90 groups compared to the pre-treatment point. SAK90's ability to improve health is also demonstrated by its propensity to lower the death rate (20%) of mice receiving SAK90. This value was 2.25 times lower than the value of the DMBA control group (45%) (Figure 1). The initial benefit of the medication in lowering cancer mortality has been demonstrated, even though the reduction was not statistically significant at this time. The aforementioned findings partially suggested SAK90's capacity to enhance health following exposure to detrimental environmental agents.

In terms of hematological parameters, DMBA-induced mice without treatment had significantly higher levels of white blood cells than normal animals (Table 2). This alteration is consistent with the results of several other studies.[9,10] An increase in antibody production, which aids in the host's survival and recuperation, is probably correlated with an increase in the WBC count. With the test product's assistance, the WBC count was seen to have nearly returned to normal after the treatment period.

It is well known that DMBA directly damages liver cells by causing the generation of reactive oxygen species (ROS), DNA adducts, and changes in the activity of enzymes involved in xenobiotic metabolism phases I and II as well as enzymatic antioxidants.[11] In the current study, the DMBA control group showed a significant decrease in serum albumin concentration, along with a propensity for serum liver biomarker parameters, namely serum ALT and AST levels to rise (Table 2). These negative changes in hepatic serum biomarkers are indicative of liver injuries. Transaminase levels tended to drop and albumin concentration tended to increase concurrently in both SAK90 treatment groups. The effective restoration of liver biomarker parameters reflects SAK90's protective effects against DMBA-induced liver toxicity in mice.

According to the data in Tables 3 and 4, after 16 weeks of first-dose DMBA administration, the experimental mice in all groups developed malignancy in 23.26% (10/43 total surviving DMBA-exposed mice). Compared to the DMBA control group, the tumor incidence was lower in both SAK90 groups. Additionally, the DMBA-treated animals that received daily oral supplementation of SAK90 had a lower tumor yield (the average number of tumors per surviving mouse) than the DMBA control mice. However, SAK90 induced no positive alteration in tumor burden. Cancers were discovered not only in the liver, which is the primary site of DMBA metabolism, but also in the skin, mesentery, lung, esophagus, and kidneys. Squamous cell carcinoma (found in the skin and esophagus) and non-Hodgkin's lymphoma (a class of malignant neoplasms arising from lymphoid tissues) are the two most frequent neoplasms detected by microscopic examination. These histopathological analyses agree with what some authors have observed about common types of cancer when DMBA is present.[7,12,13]

The above findings showed that SAK90, when administered at doses of 15 mL/kg/day and 45 mL/kg/day for 16 consecutive weeks,

initially exhibited anti-cancer effects in DMBA-induced mice. These helpful effects include increased body weight, reduced mortality rate, and reduced tumor incidence and yield. Besides, SAK90 could recover from liver damage caused by DMBA. These benefits of SAK90 can be partly explained by the anti-cancer properties of the herbal ingredients including *Hedyotis diffusa*, *Scutellaria barbata*, *Perilla frutescens*, and *Wedelia chinensis* which have been demonstrated in many studies. Several compounds of these plants, such as organo-sulfur compounds, terpenoids, polyphenols, alkaloids, saponins, phenylethanoids, coumarins, and polysaccharides have been proposed to exhibit anticancer effects. In general, apoptosis, cytotoxicity, cell cycle arrest, mitochondrial suppression, reduction of DNA damage, vital enzyme inhibition, and/or blocking angiogenesis were considered as the proposed mechanisms for the anticancer potential of the studied plants.[5]

IV. CONCLUSIONS

In conclusion, our experimental results show the potential antitumor effect of SAK90 at dosages of 15 and 45 mL/kg b.w. in animal cancer models. SAK90 is an example of an herbal mixture extract that requires further studies for detailed anticancer mechanisms and, more importantly, to be developed into an adjunct agent for conventional anticancer therapy.

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