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Original Research Article

Elevated levels of serum glucose, triglyceride, and liver enzymes in a rat model of 7,12 dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis

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Abstract

Background: Uncontrolled cell proliferation in cancers has a high requirement of energy and biosynthetic substrates. Glucose and triglycerides are the main source of energy as well as the primary building blocks for forming cellular components of mammalian cells.

Objective: This study aims to evaluate the shifting of serum glucose, triglyceride, and nitrogen wastes in the form of urea and creatinine levels; and liver enzymes levels, alanine transaminase (ALT) and aspartate aminotransferase (AST), in rat model carcinogenesis with a single dose of 7,12-dimethylbenzanthracene (DMBA) for 16 weeks observation..

Methods: The experimental animals of Rattus norvegicus strain Sprague Dawley were divided into two groups, namely the control group and the DMBA-induced group. A blood chemistry examination was carried out at weeks 4, 8, 12, and 16 post-induction using a spectrophotometer. In addition, observation of breast tumor formation and histological examination of the tumor and organs, including liver, lung, intestine, and kidney, were performed to confirm cancer formation.

Results: Five of the six experimental rats (83.3%) induced by DMBA experienced breast and lung cancer formation accompanied by continuous weight loss starting at week 10 after induction. Serum glucose levels increased significantly at weeks 12 and 16 after induction, while serum triglyceride, ALT, and AST levels increased significantly from week 4 after induction until the end of the experiment. Serum urea levels did not show a significant difference from the control group. Nonetheless, creatinine decreased at the last examination.

Conclusion: Elevated serum glucose, triglycerides, ALT, and AST levels accompanied the chemical carcinogen-induced cancer development. Studies at the clinical level are needed to prove whether abnormally elevated of these blood chemistry levels can be used to detect the presence of cancer early.

Keywords: *DMBA; breast cancer; lung cancer; cancer metabolism.*
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INTRODUCTION

Tumors require supporting systems, including metabolism, which plays a role in supplying energy and biosynthetic materials for uncontrolled cell growth.[1] With its unique metabolic characteristics, both the Warburg effect and other forms of metabolic reprogramming create a distinctive metabolic interrelationship in the body of cancer patients.[1-3]

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It is not clear how metabolic pathways contribute to each stage of carcinogenesis. However, it can be predicted that each stage has different energy requirements and biosynthetic materials.

Glucose and triglycerides are the main metabolic substrates circulating in the blood. These substrates also play an important role in supporting tumor growth.[3,4] As is well known, cancer cells show dependence on glucose (Warburg effect) as an energy source as well as a precursor for biomass formation.[3] On the other hand, several studies indicate an association between hyperglycemia-related conditions, especially diabetes mellitus, obesity, pancreatitis, and chronic stress with tumorigenesis.[5] Several types of cancer show an increase in de novo synthesis of fatty acids required for membrane biogenesis, energy production, protein modification, and signaling.[4,6] The increase in fatty acid synthesis will be subsequently related to the synthesis of triglycerides. Previous studies have shown that breast cancer patients with metastases had higher glucose and triglyceride levels than patients without metastases and controls.[7]

The liver is the main metabolic organ that regulates energy metabolism. It is not known how changes in metabolic interrelationships in cancer affect the liver and whether routine liver function biomarkers such as alanine transaminase (ALT), aspartate transaminase (AST), and gamma-glutamyl transferase (GGT) can describe cancer development so that they have diagnostic and or prognostic value. However, several studies have shown that this routine hepatic biomarker has prognostic significance in various non-liver originating diseases such as diabetes type II and cardiovascular diseases.[8] So, it should be expected that this biomarker also has biomedical significance in cancer.

This study investigated whether the main energy and biosynthetic substrate levels, glucose and triglyceride, along with nitrogen waste levels in the form of urea and creatinine, changed in blood during cancer development in a rat model. In addition, blood ALT and AST levels were also evaluated to represent the impact of cancer development on liver function.

MATERIALS AND METHODS

Research design

This study is an experimental study on animal models with a pretest-repeated posttest control group design. The animal handling and experimentation protocol of this study has been approved by the Animal Ethics Committee from the Faculty of Medicine Ethics Commission, Hasanuddin University, with letter number 455/UN4.6.4.5.3L/ PP361 2019 and followed the International Guiding Principles for Biomedical Research as developed by the Council for International Organization of Medical Sciences (CIOMS). The research was carried out at the Animal Laboratory and Biochemistry Laboratory, Faculty of Medicine, Hasanuddin University, in July 2019 to June 2020.

Experimental animals

This study used white rat Rattus norvegicus strain Sprague Dawley aged 8 weeks with a bodyweight ranging from 100-150 g obtained from the Faculty of Agriculture, Livestock, and Fisheries, Muhammadiyah University of Pare-Pare, South Sulawesi. Arriving at the Biochemistry Laboratory, Faculty of Medicine, Hasanuddin University, the animals were acclimatized for one week before undergoing the experiment. Animals are housed individually, free access to rodent chow and drink (ad libitum). The temperature of the maintenance room was around 25 ± 2 °C with a humidity of 50-55% in a cycle of 12 hours light and 12 hours dark. The cages were regularly cleaned every day. Rats were weighed every week. By referring to the calculation of the number of experimental animals using the resource equation approach[9], the rats were grouped into the control group, which consisted of 5 rats, and the group induced with DMBA, which consisted of 6 rats (the minimum number was used in the control group, and the maximum number was used in the treatment).

Cancer induction using DMBA

Each rat in the induction group received a single dose of 25 mg of DMBA (Sigma Chemicals), which was dissolved in a mixture of 0.5 mL of sunflower oil and 0.5 mL of 0.9% NaCl and pre-warmed to 37°C. DMBA solution was administered using an intragastric gavage. Rats in the control group received a mixture of these solutions without DMBA with the same route of administration. Palpation of the rat breast gland areas was carried out every week to check for tumor formation. Blood samples were taken before induction, at weeks 4, 8, 12, and 16 post-induction to measure serum glucose, triglyceride, ALT, AST, urea, and creatinine levels. Before the blood draw, feeding was stopped for 12 hours. Rats were euthanized at week 16 for tumor removal and organ harvesting, including liver, lungs, intestines, and kidneys. Tumor tissues and organs of interest were immersed in 10% formalin solution and sent on the same day to the Anatomical Pathology Laboratory, Hasanuddin University Hospital, for histopathological examination.

Measurement of blood glucose, triglycerides, ALT, AST, urea, and creatinine levels

Measurements of these blood chemistry levels followed the protocol kit produced by Quimica Clinica Aplicada, Spain, and read using a Genesys 150 UV VIS spectrophotometer from Thermo Scientific.

Histopathological examination

Tumor tissues and harvested organs were processed into formalin-fixed, paraffin-embedded sections and then stained with hematoxylin and eosin (HE). A pathologist evaluated histological abnormalities of the tissue that performed blindly, without knowing what treatment was given to each tissue preparation.

Statistic test

All values presented are mean ± SEM with the p-value of mean differences between groups tested using the student T-test in Microsoft Excel 2016.

Table 1. Summary of the observation of tumor formation in DMBA-induced carcinogenesis rats

Observed items	DMBA 1	DMBA 2	DMBA 3	DMBA 4	DMBA 5	DMBA 6
Time of breast tumor mass was firstly palpable	Week 9	Week 8	Week 9	-	Week 12	Week 9
Number of tumor nodules	1	1	1	-	1	1
Ulcerative tumor	-	-	+	-	-	+
Lung carcinoma (by histologic examination)	+	+	+	-	-	+
Carcinoma in liver, intestine, and kidney	-	-	-	-	-	-
Survive to week 16	√	√	√	√	√	√

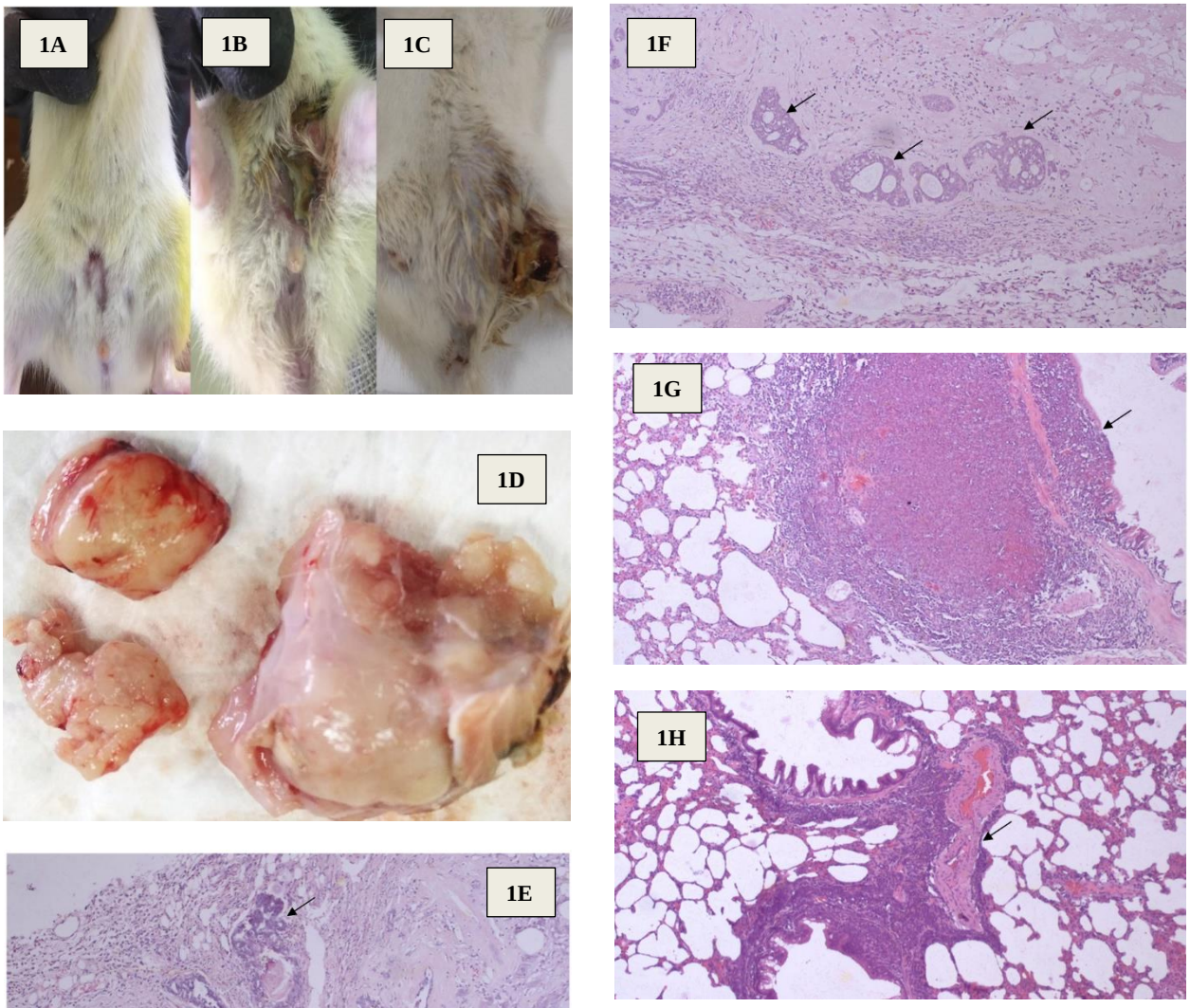

Figure 1. Tumor formation in experimental rats after DMBA induction. Observation of tumor formation in the breast area at weeks 8, 12, and 16 (A, B, C, respectively). Representation of rat breast tumor masses after being euthanized at week 16 (D). The representation of histological examination of breast tumors (E, F) and lungs (G, H) showed the formation of carcinoma (indicated with black arrows)

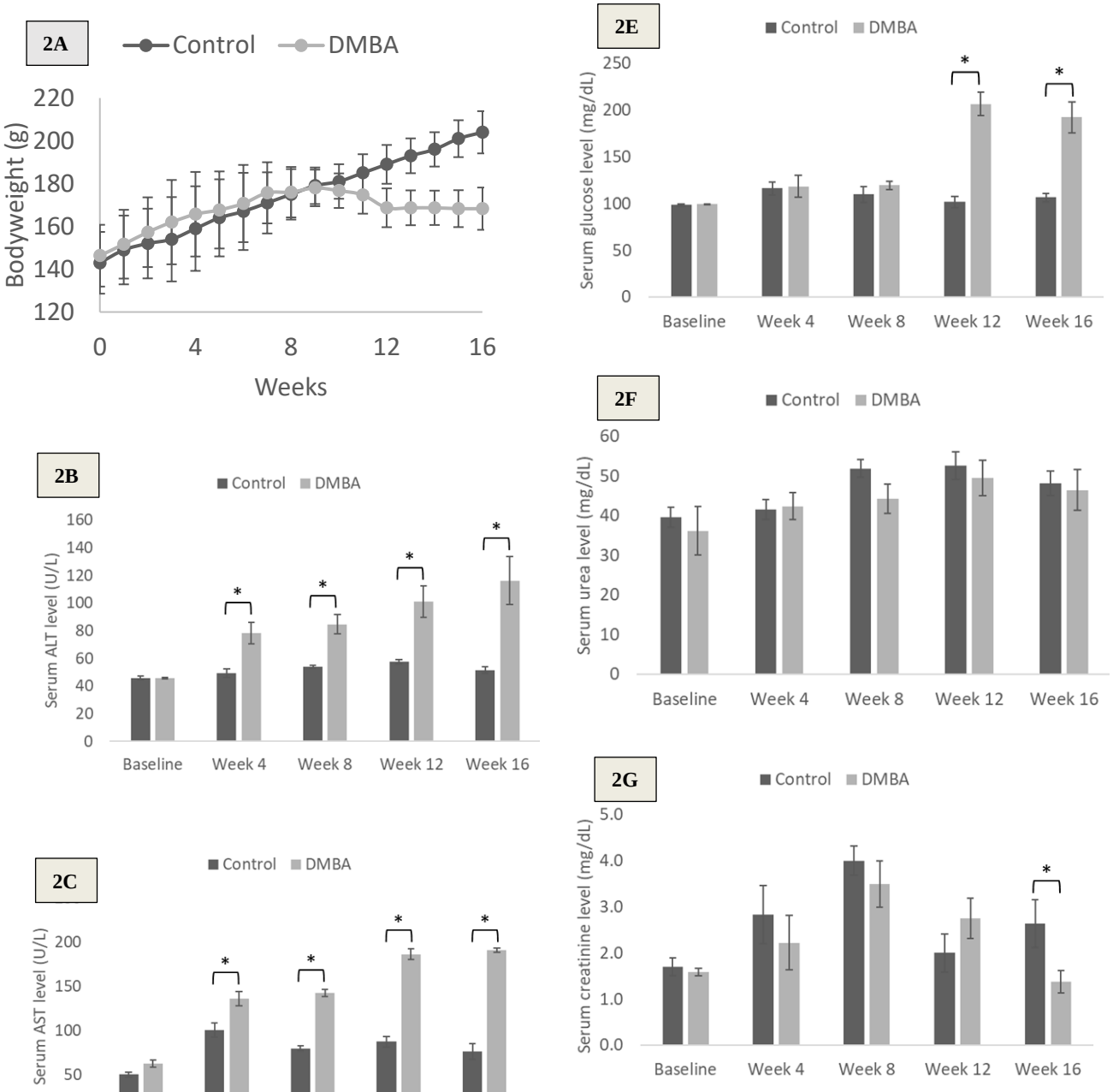

Figure 2. Development of rat bodyweight (A) and blood chemistry examination of ALT, AST, triglyceride, glucose, urea, and creatinine (B, C, D, E. F, G, respectively). Data are presented as mean ± SEM. *Student T-test showed a significant difference between the control and DMBA-induced groups.

RESULTS

DMBA induced breast and lung cancer formation

Tumor development was characterized by forming a solid mass in the breast area (Table 1). Dense mass was observed in one of the 6 rats in the DMBA induction group at week 8, at week 9 and week 12 it was added by three rats and one rat, respectively (Figure 1A-D). Until week 16, one DMBA-induced rat did not form nodules in the breast area at all. Two out of five rats had breast tumors that developed into ulcers (fungating cancer wounds). Histological examination showed invasive breast carcinoma no special type for all nodules examined. In addition, lung histology examination

showed that four of the six DMBA-induced rats exhibited lung carcinoma (Figure 1E-H). Histological examination of the livers, intestines, and kidneys did not find any abnormalities or spread of cancer cells. No tumor formation was found in the control group.

DMBA induction group showed weight loss

There was an increase in the bodyweight of rats every week in both groups until week 9. However, when entering week 10, weight loss began to occur in the DMBA induction group, although significant weight loss began to be observed in week 13 (Figure 2A).

Increased levels of serum glucose, triglycerides, ALT, and AST in the DMBA induction group

Serum glucose levels in the DMBA induction group were approximately two times higher than the control group at week 12 ($p < 0.001$) and remained high until week 16 ($p = 0.007$) (Figure 2B). Serum triglyceride levels in the DMBA induction group increased by 70.55% compared to the control group starting at week 4 ($p=0.038$) and increased by more than doubled compared to the control group at week 8 ($p=0.025$), week 12 ($p=0.004$), and week 16 ($p=0.002$) (Figure 2C). The serum ALT and AST levels of the DMBA induction group increased gradually since week 4, while the control group tended to be constant (Figure 2D). Urea and creatinine did not significantly differ between the two groups (Figures 2E and 2F). However, a decrease in creatinine levels was found in the DMBA group when entering week 16 (Figure 2F).

DISCUSSION

Cancer formation is thought to be due to 90% of chemicals, 5% of radiation, and 5% of viruses. All chemical carcinogens or their derivatives are highly reactive electrophiles that can attack nucleophilic, electron-rich sites in the cell, especially deoxyribonucleic acid (DNA), to form adducts through one or more covalent bonds and subsequently cause mutations.[10] DMBA is a synthetic polycyclic aromatic hydrocarbon (PAH) that has long been used as a mutation-inducing agent in cancer research such as breast, lung, lymphoid tissue, intestine, and skin cancer.[11] Intragastric administration of DMBA will be followed by the hepatocytes uptake of this substance. Through xenobiotic metabolism mediated by cytochrome P450, CYP1B1, and microsomal epoxide hydrolase, these compounds are converted to a proximate carcinogenic metabolite, DMBA-3,4-diol. Furthermore, by CYP1A1 or CYP1B1, this compound is further oxidized to form the principal ultimate carcinogenic metabolite, DMBA-3,4-diol-1,2-epoxide.[12] From the liver, these carcinogenic metabolites then circulate throughout the body, interacting with rapidly proliferating cells, forming DNA adducts and subsequent mutations, which are the initial stage of cell transformation to become malignant.[12-14] In this study, after 16 weeks of single-dose administration of DMBA, it resulted in 83.33% formation of malignancy in the mammary glands and lungs of the experimental rats. Malignancies were not found in the liver, which is the main site of DMBA metabolism, nor in the intestines and kidneys.

DMBA is known to have direct toxic effects on liver cells through the production of reactive oxygen species (ROS), formation of DNA adducts, and influencing the activity of enzymes that play a role in the phase I and II of xenobiotic metabolism and enzymatic antioxidants.[12-14] Although there were no signs of malignancy in liver tissue on histologic examination, this study showed an increase in serum liver enzymes, ALT and AST, gradually starting from 4 weeks post-induction until the last examination at week 16. These results are in line with the research of Arora et al. using the animal model Rattus norvegicus strain Wistar.[15] This present study also showed an increase in serum triglyceride levels, which began to occur in week 4 of examination after DMBA induction. The study conducted by Li Fang et al. showed that intratracheal administration of PAH, benzo[α]pyrene (B[α]P), caused disturbances of lipid metabolism in the liver, both uptake and synthesis, including triglycerides, leading to non-alcoholic fatty liver disease (NAFLD).[16] As a form of PAHs, DMBA most likely affects the increase in serum triglyceride levels by the same mechanism as B[α]P. While a study by Isimura Y, et al. showed that oral administration of the amine heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4-5-b]pyridine (PhIP) induces hypertriglyceridemia in F344/DuCrj rats.[17] Further investigations are needed regarding the increase in triglyceride levels after administration of carcinogens to determine its contribution to cancer development.

Glucose is the main substrate needed by rapidly proliferating cancer cells (Warburg effect). So it is understandable if hyperglycemia promotes cancer development. Studies have shown a high cancer incidence in diabetes mellitus type 2.[5,18] This present study showed an increase in glucose in weeks 12 and 16 of the experiment. The limitation of this study was that it did not examine the pancreas to determine the toxic effect of DMBA on this organ which could be a rationale for an increase in glucose levels. However, these increased glucose levels can be associated with metabolic interrelationships in carcinogenesis. Studies identified proteolysis inducing factor (PIP) and lipid mobilizing factor (LMF) that stimulate muscle protein catabolism and lipid mobilization from adipose tissues.[19] Another study showed that there was an increase in the activity of key gluconeogenesis enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and fructose-1,6-bisphosphatase 1 (FBP1), which is a form of metabolic reprogramming to meet the excess demand for glucose in cancers.[20,21] This evidence is mutually reinforcing as justification that the increase in serum glucose levels in carcinogenesis occurs due to increased activity of gluconeogenesis with substrates derived from muscle proteolysis and lipolysis. Mobilization of lipids from adipose tissue may also be a logical consequence of elevated serum triglycerides apart from the direct effect of DMBA on lipid metabolism. Furthermore, the induction of protein catabolism and mobilization of fat from adipose tissue is the basic mechanism of weight loss to cachexia in cancer, as shown in the rats' weight loss when entering week 10 of the experiment. The decrease in serum creatinine levels shown at the end of the experiment can be an indicator of decreased muscle mass due to cachexia.

CONCLUSION

Serum glucose, triglyceride, ALT, and AST levels increased along with tumor development induced by the carcinogenic chemical, DMBA. So, this study indicated that DMBA as a type of PAH carcinogens may induce carcinogenesis accompanied by changes in glucose metabolism and lipid metabolism, especially triglycerides, which promote the further development of cancer. PAHs are atmospheric pollutants widely distributed in the environment due to incomplete combustion of organic materials such as coal, oil, gasoline, and wood and are even detected in cooked food.[22] Continuous exposure to these carcinogens causes those who are at risk to be susceptible to developing cancer later in life. Therefore, this study led to the notion that elevated levels of liver enzymes, glucose, and triglycerides could be an early warning for susceptible subjects to undergo further tests to rule out the possibility of the growth of cancer cells in the body.

CONFLICT OF INTEREST

The authors affirm no conflict of interest in this study.

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