Original Research Article

Effect Of Rosemary Leaf Ethanol Extract (RosmarinusOfficinalis L.) On Arginase-1 And Tumor Suppressor P53 Expression Of Hepatocellular Carcinoma In Male Wistar Strain Rats (RattusNorvegicus L.) Induced By p-Dimethylamino benzaldehyde (DMBA)

ABSTRACT

Aims: *Tumor Suppressor p53* is a frequent research target to further understand the mechanisms of cancer development and the pathogenesis of cancer. *Arginase-1* may also be a potential prognosis predictor for HCC patients, and also be a new target for HCC treatment. This research aims to scientifically test whether the administration of ethanol extract from Rosemary leaves (*Rosmarinusofficinalis L*.) inhibits the increase in *Arginase-1* and inhibits the increase in *Tumor Suppressor p53* from *Hepatocellular Carcinoma* in Rats *(Rattusnorvegicus L.)* Male Wistar induced by *p -Dimethylamino Benzaldehyde (DMBA).*

Study design: This study used an in vivo laboratory test method. This study employed true experimental laboratoriesto evaluate the effects of rosemary leaf extract.

Place and Duration of Study: Department of Pharmacology and Therapy and Department of Histology, Faculty of Medicine, Udayana University, between December 2023to July 2024.

Methodology: Wistar rats were induced with p-Dimethylamino Benzaldehyde (DMBA) and treated with varying doses of ethanol extract from rosemary leaves (200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW). A control group was included, and the experiment was replicated five times. Hepatocellular carcinoma in liver tissue samples was evaluated through immunohistochemistry for Arginase-1 and Tumor Suppressor p53 expression. Interpretation of immunoreactivity was based on Histochemical scoring (H-score) assessment.

Results: The results showed that the ethanol extract of rosemary leaves inhibited the decrease in Tumor Suppressor p53 expression in the DMBA-treated group. Significant increases in p53 expression were observed at doses of 400 mg/kg and 800 mg/kg BW. One-way ANOVA of the immunohistochemical tests for Arginase-1 and p53 revealed a significant difference between groups ($p < 0.05$).The ethanol extract of rosemary leaves demonstrated a potential to inhibit the decrease in Tumor Suppressor p53 expression but did not significantly affect the expression of Arginase-1 in hepatocellular carcinoma in male Wistar rats induced by p-Dimethylamino Benzaldehyde (DMBA).

Conclusion: Ethanol extract of rosemary leaves could not inhibit the increase in arginase-1 expression and inhibit the decrease in tumor suppressor p53 of hepatocellular carcinoma in male Wistar rats induced by p-Dimethylamino benzaldehyde (DMBA).

Keywords:Tumor Suppressor p53, Arginase-1, DMBA, Rosemary leaf

1. INTRODUCTION

The death rate from liver cancer in the United States appears to have increased by about 40% from 1990 to 2004, in contrast to the overall cancer death rate, which decreased by about 18% over the same period [1]. The worldwide occurrence of primary liver cancer is steadily rising, with projections indicating that over one million individuals will be diagnosed with the disease each year by 2025 [2]. Hepatocellular carcinoma (HCC) ranks as the fifth most prevalent cancer globally and the fourth most common among men in South Korea, where chronic hepatitis B infection is widespread among middle-aged and older individuals [3]. Hepatocellular carcinomas vary widely and are not uniform tumors, they exhibit diverse layers of heterogeneity [4]. The discovery of effective biomarkers for monitoring and early diagnosis of HCC

remains inadequate. Current serum biomarkers exhibit low sensitivity and inconsistent specificity, even when evaluated over time or used in combination with other markers, despite employing varying cut-off points [5]. Early detection is crucial for hepatocellular carcinoma (HCC) because an early diagnosis can greatly enhance the prognosis, which is closely linked to the tumor stage [6]. Liver cancer shows extensive diversity in its development, histopathological traits, and biological behavior. This variation in causative factors significantly contributes to the generally unfavorable prognosis seen in liver cancer patients [7]. The classification of hepatocellular carcinoma (HCC) is mainly based on its morphological features, with histopathological evaluation serving as the gold standard for diagnosing HCC and differentiating it from other possible conditions [1].

L-arginine is a positively charged amino acid that plays a role in various metabolic processes [8]. Arginase 1 (ARG1) and arginase 2 (ARG2), the two mammalian arginase isoforms, are significantly overexpressed in various cancers. They contribute to tumor growth and metastasis through several mechanisms, such as altering L-arginine metabolism and influencing the tumor immune microenvironment [9]. Arginase 1 has a significant role in the cancer microenvironment (tumor microenvironment) and is also a new target for the treatment of HCC [10].

The normal function of tumor suppressor p53 is a powerful barrier to cancer. The tumor suppressor protein p53 plays a key role in regulating the cell cycle and responding to cellular stress, such as exposure to carcinogens (carcinogenic agents) [11]. Loss of normal p53 function due to mutations can allow uncontrolled growth of cancer cells and is a major contributing factor in the development of cancer. Tumor Suppressor p53 is a frequent target of research in an effort to better understand the mechanisms of cancer development and potential cancer therapies. Disruption of the p53 tumor suppressor signaling pathway overcomes the apoptosis checkpoint and causes further cell division in hepatocytes with already shortened telomeres until the telomeres become very short. At this point, the cells enter a crisis checkpoint, characterized by massive cell death. Insufficient telomerase activity leads to accelerated telomere shortening in proliferating liver cells and as a result, genomic instability. Telomere shortening is an important risk factor for tumor development in liver carcinogenesis. The risk of tumor formation increases dramatically in the cirrhosis stage, which is characterized by increased apoptosis in hepatocytes, and further cell division in the crisis stage through hepatocyte apoptosis [7].

Anticancer therapy for hepatocellular carcinoma is currently not as much as other anticancer therapies. One of the recommended first-line anticancer drugs for hepatocellular carcinoma is Sorafenib and Lenvatinib. These drugs have various limitations in the side effects they cause and drug resistance often occurs. Side effects occur because the selectivity of the drug works at many drug target sites [12]. This problem underlines the importance of developing anticancer drugs for therapy in hepatocellular carcinoma.

Medicinal plants that are efficacious in cancer therapy are rosemary. Rosmarinic acid has also been studied in animal models of Hepatocarcinoma with biomarkers TNF-α, TGF-β and IFN-γ [13]. In addition, there has been no testing and development of this compound as an antitumor drug on the expression of Arginase-1 and tumor suppressor p53.

Potential as a useful material as an anticancer and phytochemicals taken for example phenol and flavonoids. Rosmarinic acid has solubility can dissolve in ethanol so this study uses ethanol extract as a material in its testing. Rosmarinic acid is a compound that can be found in nature especially in rosemary leaves. Several studies have confirmed the various therapeutic benefits of rosmarinic acid in various diseases, including cancer, diabetes, inflammatory disorders, neurodegenerative disorders, and liver disease. Rosmarinic acid is a bioactive phenolic compound commonly found in plants of the Lamiaceae and Boraginaceae families. Rosmarinic acid is biosynthesized using the amino acids tyrosine and phenylalanine through reactions catalyzed by enzymes.

This study aims to scientifically test the administration of rosemary leaf ethanol extract in inhibiting the increase in arginase-1 expression and inhibiting the decrease in tumor suppressor p53 from hepatocellular carcinoma in male Wistar rats (Rattusnorvegicus L.) induced by p-Dimethylamino benzaldehyde (DMBA).

2. MATERIAL AND METHODS

2.1 MATERIALS

The tools used in this study include analytical scales, stainless spoons, ovens, *object glasses* , *cover glasses* , droppers, ratscages , rats drinking bottles, husks, SPSS 26 software , rats food, gloves, masks, 1 cc and 3 cc syringes, probes, rats fixation tools, ketamine, data loggers, cameras, microscopes , styrofoam boxes, plastic tissue pots, plastic bottles, scalpels, tweezers, strainers, tissue cassettes, automatic processor machines, vacuum machines, blocking machines, microtome machines, microtome knives, 46 ^{o}C water baths, object glasses, cover slips, special staining racks, 60 ^{o}C ovens.

2.2 METHODOLOGY

The type of research used is true experimental laboratories in vivo. The research design applied for oral administration of rosemary leaf ethanol extract (Rosmarinusofficinalis L.) inhibits hepatocellular carcinoma levels in this study is a post test only control group design.

2.3 SAMPLE

In this study, specimens were collected from rat that met the inclusion and exclusion criteria with the following provisions:

2.3.1 INCLUSION CRITERIA

a. Male Wistar Rat (Rattusnorvegicus L.)
b. Rat weight 100-250 grams
c. Healthy rats aged 2-3 months.

2.3.2 EXCLUSION CRITERIA

a. Rats don't want to eat or drink
b. Rats died and liver tissue could not be taken.

2.4 PROCEDURES

This study is a true experimental laboratories in vivo. This research protocol has met the ethical principles of research, the research protocol was reviewed and approved by the Udayana University ethics committee with a letter or Ethical Clearance document Number: B / 259 / UN14.2.9 / PT.01.04 / 2023. This research protocol has received approval for the implementation of research from the Integrated Biomedical Laboratory with a letter or document Number: 1568 / UN14.2.2.VII.6 / LT / 2023. The total sample of each group was 7 rats. The total number of samples was 35 rats.

a. Control negatifgroup and treatment group of rats were induced with DMBA twice a week for 5 weeks at a dose of 25 mg/kg BW. DMBA was administered orally using a probe. DMBA was dissolved in corn oil in a Bio Safety Cabinet for the carcinogenic induction process.
b. Normal rat group and DMBA-induced rat group were given pellets and drink ad libitum during the treatment process. The waiting period for cancer growth was 2 weeks.
c. Group of rats that met the inclusion criteria with DMBA induced treatment of 25 mg/kg BW for 2 times a week for 5 weeks. Waited for 2 weeks for tumor formation with treatment given food and water ad lib during the study. Treatment group continued by giving rosemary leaf ethanol extract at a dose of 200 mg/kg BW , 400 mg/kg BW, 800 mg/kg BW once a day for 20 days
d. Final treatment all rats (Rattusnovergicus L.) were sacrificed with ketamine. Liver organ was taken for Immunohistochemistry measurement of Tumor Suppressor p53 expression.

3. RESULTS AND DISCUSSION

The organoleptic properties of the rosemary leaf ethanol extract were visually assessed. It was observed that the extract had a blackish-brown color. When exposed to air and light at room temperature, the extract absorbed oxygen, resulting in a darker color. Phytochemical screening tests were conducted with the aim of determining secondary metabolite compounds and bioactive components contained in the ethanol extract of rosemary leaves.

Table 1. Results of the Normality Test for Body Weight of Research Rats

Sampel	Fenol (mg/100g)	Flavonoid (mg/100g)	IC 50 (ppm)	Antioxidant capacity (mg/L GAEAC)
Ekstrak*Rosemary*	4880,70	51348,04	216,0088	4452,90

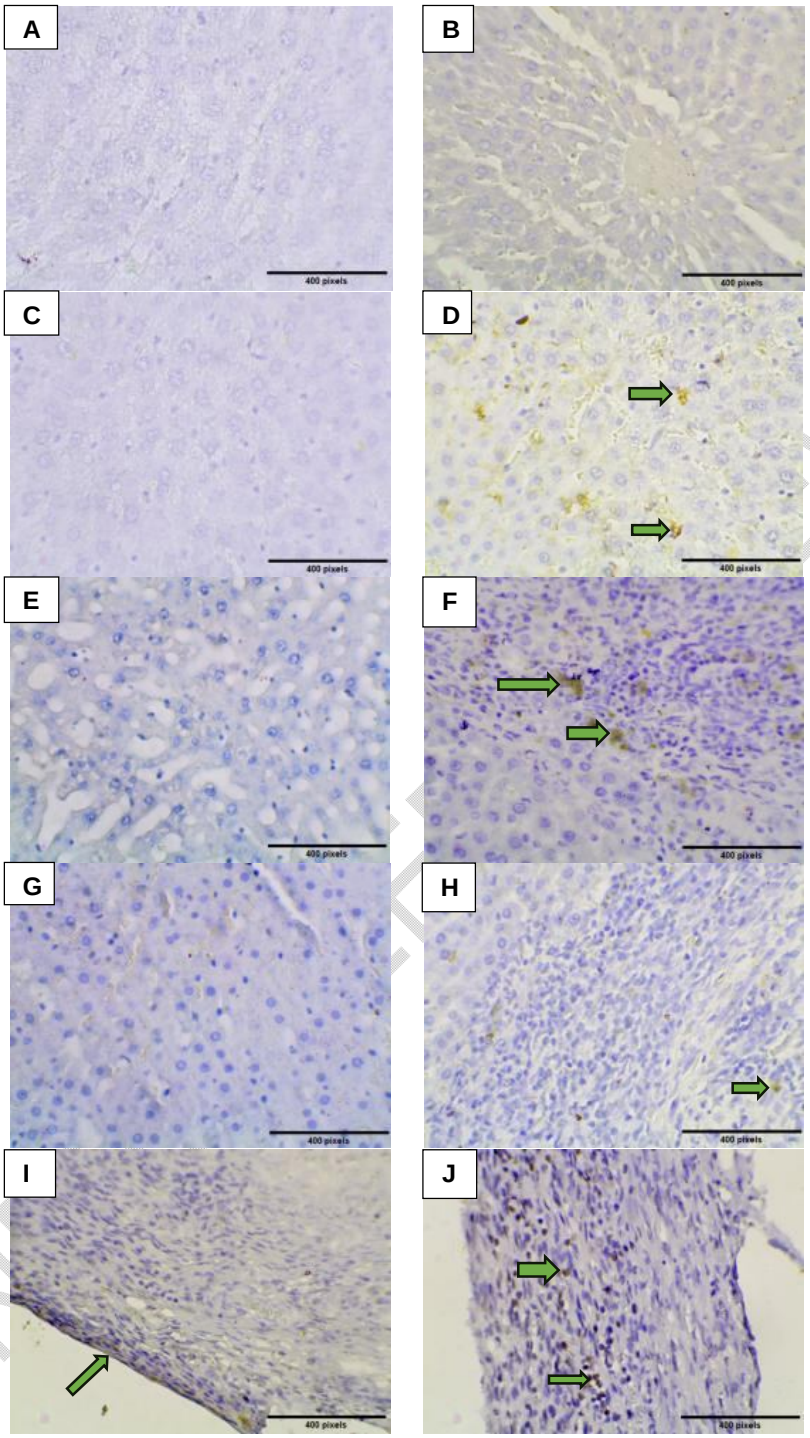

Fig 1. Arginase-1 expression and p53 wild type expression compare to normal liver and control negative sample.
A. NegativeArginase-1 expression from normal liver. B.Negativep53 wild type expression from normal liver. C. Negative Arginase-1 expression from control negative sample. D.Negative p53 wild type expression from control negative sample. E. Negative arginase-1 expression from liver DMBA induced with treatment rosemary leaf ethanol extract at a dose of 200 mg/kg BW. F. Negative p53 expression from liver DMBA induced with treatment rosemary leaf ethanol extract at a dose of 200 mg/kg BW. G. Negative arginase-1 expression from liver DMBA induced with treatment rosemary leaf ethanol extract at a dose of 400 mg/kg BW.H. Negative p53 wild type expression from liver DMBA induced with treatment

rosemary leaf ethanol extract at a dose of 400 mg/kg BW. I. Positive arginase-1 expression from liver DMBA induced with treatment rosemary leaf ethanol extract at a dose of 800 mg/kg BW. J. Positive p53 wild type expression from liver DMBA induced with treatment rosemary leaf ethanol extract at a dose of 800 mg/kg BW.

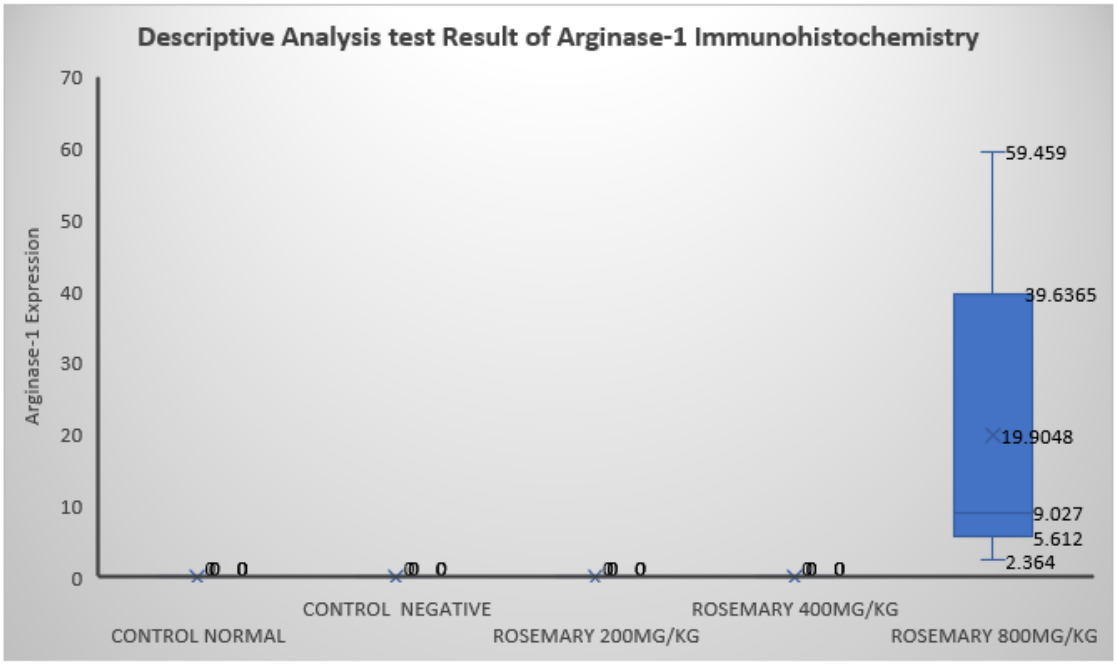

Fig. 2Descriptive analysis results of Arginase-1 ImmunohistochemistryH-score Data(indicates negative arginase-1 expression and abnormal data from group control normal, control negative, Rosemary 200mg/kg BW, Rosemary 400mg/kg BW with P-value < 0.001, indicates positive arginase-1 expression and normal data from rosemary 800mg/kg BW group with P-value >0.01).

Table 2. Results of the Homogeneity Test and One-way ANOVA Test Results of Arginase-1 Immunohistochemistry H-score Data

Variable	Levene statistic	*p*	Result	One-way Anova *p*	Result
ARG 1 Expression	2,083	0,247	Homogen	0,02	Significant differences

Table 3. Post Hoc Test Results with Least Significantly Different (LSD) H-score Data of Arginase-1 Immunohistochemistry

Variable		*p*	Result
Normal Control	Negative Control	1,000	Not Significant
Normal Control	*Rosemary* 200mg/kg BW	1,000	Not Significant
Normal Control	*Rosemary* 400mg/kg BW	1,000	Not Significant
Normal Control	*Rosemary* 800mg/kg BW	0,006	Significant differences
Negative Control	*Rosemary* 200mg/kg BW	1,000	Not Significant
Negative Control	*Rosemary* 400mg/kg BW	1,000	Not Significant
Negative Control	*Rosemary* 800mg/kg BW	0,006	Significant differences
Rosemary 200mg/kg BW	*Rosemary* 400mg/kg BW	1,000	Not Significant
Rosemary 200mg/kg BW	*Rosemary* 800mg/kg BW	0,006	Significant differences
Rosemary 400mg/kg BW	*Rosemary* 800mg/kg BW	0,006	Significant differences

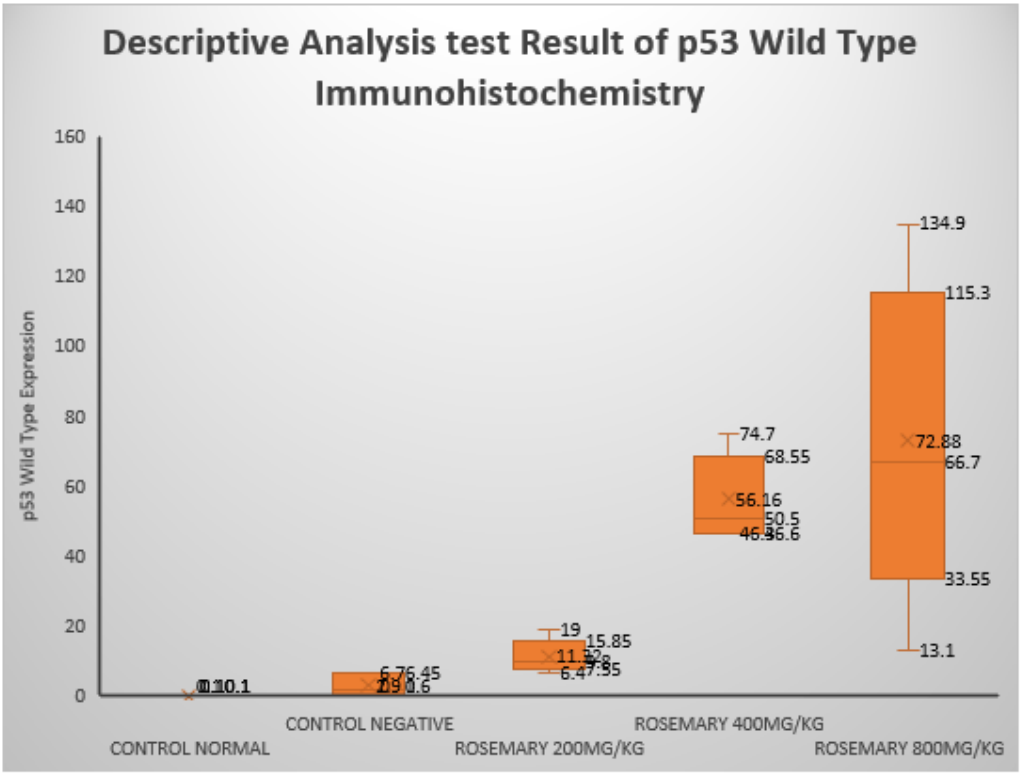

Fig. 3 Descriptive analysis results of p53 Wild Type Immunohistochemistry H-score Data (indicates negative p53 Wild Type expression and abnormal data from group control normal with P-value < 0.001, indicates positive p53 Wild Type expression and normal data from control negative, Rosemary 200mg/kg BW, Rosemary 400mg/kg BW, Rosemary 800mg/kg BW group with P-value >0.01).

Table 4. Results of the Homogeneity Test and One-way ANOVA Test Results of p53 wild type Immunohistochemistry H-score Data

Variable	Levene statistic	*p*	Result	One-way Anova *p*	Result
P53 wild type Expression	4,935	0,053	Homogen	0,000	Significant differences

Table 5. Post Hoc Test Results with Least Significantly Different (LSD) H-score Data of p53 wild type Immunohistochemistry

Variable		*p*	Result
Normal Control	Negative Control	0,837	Not Significant
Normal Control	*Rosemary* 200mg/kg BW	0,415	Not Significant
Normal Control	*Rosemary* 400mg/kg BW	0,000	Significant differences
Normal Control	*Rosemary* 800mg/kg BW	0,000	Significant differences
Negative Control	*Rosemary* 200mg/kg BW	0,539	Not Significant
Negative Control	*Rosemary* 400mg/kg BW	0,001	Significant differences
Negative Control	*Rosemary* 800mg/kg BW	0,000	Significant differences
Rosemary 200mg/kg BW	*Rosemary* 400mg/kg BW	0,003	Significant differences
Rosemary 200mg/kg BW	*Rosemary* 800mg/kg BW	0,000	Significant differences
Rosemary 400mg/kg BW	*Rosemary* 800mg/kg BW	0,229	Not Significant

Anticancer therapy for hepatocellular carcinoma is currently not as much as other anticancer therapies. Rosemary for biological activities, such as antibacterial, anticancer, antidiabetic, anti-inflammatory and antinociceptive, antioxidant, antithrombotic, antiulcerative, improving cognitive deficiency, antidiuretic, and hepatoprotective effects are the prospects for the use of rosemary as an agent in the treatment of various diseases. Rosemary has long been considered as a herb and occupies a special place in traditional medicine. Phenolic diterpenes, triterpenes, phenolic acids, such as carnosic acid (CA), carnosol, rosmanol, ursolic acid, betulinic acid, and rosmarinic acid (RA), and nepitrin are the pharmacologically active constituents identified in Rosemary. Isolated phenolic compounds in rosemary, carnosic acid and rosmarinic acid have been shown to have the most common pharmacological effects and interact with several molecular targets[14].

Plant phenolic compounds can inhibit the metabolic activation of procarcinogens, lowering the levels of highly reactive substances in cells and protecting genomic DNA from damage. These compounds also interact with factors involved in epigenetic regulation, such as miRNAs and enzymes that modify DNA and histones, whose misregulation may contribute to cancer development. Additionally, polyphenols have been shown to counteract the pro-survival and growth-promoting effects of pro-inflammatory signals on tumor cells [15].

Literature review study of existing in vitro and in vivo studies focused on the anticancer effects of rosemary extract and rosemary extract polyphenols namely carnosic acid and rosmarinic acid, and their effects on key signaling molecules. The main polyphenols found in rosemary extract include the diterpenescarnosic acid and rosmarinic acid. In vitro and in vivo studies on the effects of rosemary and its major polyphenols have been summarized and sorted by cancer cell type, in chronological order from earliest to latest. The anticancer effects of rosemary extract include several in vitro studies using colon cancer cell lines have shown rosemary to exhibit anticancer properties. Rosemary extract is summarized to have effects in increasing the tumor suppressor p53 as a proapoptotic[16].Rosmarinusofficinalis L., commonly known as rosemary, contains compounds that have shown potential in influencing various aspects of cancer biology. In particular, its main polyphenolic compounds, carnosic acid (CA) and rosmarinic acid (RA), play a pivotal role in this regard. These compounds demonstrate anticancer properties through their ability to modulate cell proliferation, induce apoptosis, inhibit angiogenesis, and suppress metastasis. Their multifaceted impact on cancer pathways highlights their potential as valuable agents in the development of novel cancer therapies [17].

In this study, a multilevel maceration method was chosen to separate the antioxidant compounds in the rosemary leaf extract based on their polarity. The main constituents of rosemary leaves, namely phenol and flavonoids, have solubility that can dissolve in ethanol solvents although they are insoluble in n-hexane and slightly soluble in ethyl acetate. The results of the analysis of rosemary leaf ethanol extract using the multilevel maceration method in this study were organoleptically in the form of a thick liquid, reddish brown in color and a distinctive odor of rosemary leaves. Testing of flavonoid, phenol, IC50 levels, and antioxidant capacity of rosemary leaf ethanol extract was carried out as confirmation of the content of secondary metabolite compounds from the rosemary leaf ethanol extract made.

The antioxidant capacity value in the ethanol extract of rosemary leaves obtained results of 4452.90 mg/L GAEAC and the IC50 value was found to be 216.0088 ppm. The IC50 value shows a concentration of 216.0088 ppm where the extract inhibits 50% of free radicals with an antioxidant capacity of 4452.90 mg/L GAEAC. The IC50 value in this study shows that a large concentration of extract is needed to achieve 50% free radical inhibition. Although, the antioxidant capacity measured in mg/L GAEAC (Antioxidant Capacity Equivalent to Gallic Acid) has a very high value, indicating strong overall antioxidant activity. The antioxidant content is increased by administering 3 high dose variants. This study used a dose of rosemary ethanol extract of 200 mg/kg BW, 400 mg/kg BW and 800 mg/kg BW.

Based on a meta-analysis of preclinical in vivo inflammation models using Rosmarinusofficinalis as therapy at a dose of 100 mg/day for 21 days [13]. Based on in vitro studies on human cancer cell lines using crude rosemary ethanol extract has differential antiproliferative effects on human leukemia

and breast carcinoma cells at a dose of 200 mg/mL [18]. The dosage variations of rosemary leaf ethanol extract used in this study were selected at 200 mg/kg BW, 400 mg/kg BW, 800 mg/kg BW.

Albino rats (Rattusnorvegicus L.), commonly used as laboratory models in biomedicine, were employed in this study. Rats can represent mammalian biological systems, making them very suitable for preclinical research. The reproductive period of rats can be determined by observing their various life stages and behaviors. The liver of Wistar rats has a structure and function similar to the liver of other mammals, used in biomedical research to study liver function, metabolism, and the effects of various compounds or drugs.

In this study, OnewayAnova test was conducted to compare various treatment groups with normal controls and between treatment groups. The Least Significant Difference (LSD) test is a post-hoc method used to perform multiple comparisons between means of several groups after performing analysis of variance (ANOVA). The LSD test is used to identify which pairs of groups have significant differences.

a. Effect of Rosemary Leaf Ethanol Extract on Arginase-1 Expression of Hepatocellular Carcinoma in Male Wistar Rats Induced by p-Dimethylamino benzaldehyde (DMBA).

Arginase, an enzyme that converts arginine into ornithine and urea, is produced by myeloid-derived suppressor cells (MDSCs), natural killer cells, and neutrophils within the tumor microenvironment (TME) and is detected in the plasma of cancer patients. The presence of MDSCs and the arginase they produce is associated with poorer clinical outcomes, as MDSCs are abundant in cancer patients and correlate with distant metastases. Arginase promotes immunosuppression in the TME by depleting arginine, which inhibits the function and proliferation of effector T cells and natural killer cells. Studies have shown that arginase knockout in macrophages reduces tumor growth, while arginine supplementation or arginase inhibition enhances antitumor T-cell responses and boosts the efficacy of immunotherapy in murine tumor models. Given its role in immunosuppression, pharmacological inhibition of arginase presents a promising approach for cancer immunotherapy. However, since arginase plays a critical role in the urea cycle to eliminate toxic ammonia, its inhibition could disrupt this cycle, potentially leading to hyperammonemia. Elevated levels of orotic acid in urine serve as a sensitive marker of urea cycle disruption. When arginase activity is impaired, the metabolic pathway diverts to pyrimidine synthesis, causing an accumulation of the pyrimidine precursor orotic acid [19].

ARG1 contributes to the development of hepatocellular carcinoma by promoting carcinogenesis. Elevated ARG1 expression is associated with more aggressive tumor growth, larger tumor size, and increased levels of alanine aminotransferase (ALT) and glutamyl transferase (GGT) [9]. Immunosuppressive macrophages are traditionally identified by the expression of the enzyme Arginase 1 (ARG1), which we observed to be highly expressed in pancreatic tumor-associated macrophages in both human patients and rats models [20].

Arginase 1 also plays a role in modulating the immune system, primarily through its effects on arginine metabolism. Arginase 1 is expressed by alternatively activated macrophages (M2) and myeloid suppressor cells (MDSCs). This enzyme catalyzes the depletion of arginine in the microenvironment, which can suppress T cell activity and facilitate anti-inflammatory or immunosuppressive responses. High expression of arginase 1 by tumor cells or stromal cells in the tumor microenvironment can lead to local immunosuppression, allowing tumors to evade detection by the immune system. Arginase 1 is considered a potential target for immune cancer therapy in this study. Decreased Arginase 1 function in chronic liver diseases, such as cirrhosis or hepatitis, may impair ammonia detoxification and slow hyperammonemia. Further study of Arginase 1 may pave the way for novel therapies in these conditions.

Arginase 1 is often used as a marker to identify specific cell types and enzymatic activities in various tissues, especially in the liver. Arginase 1 is highly expressed in hepatocytes, the primary cells of the

liver. In liver histology, immunohistochemistry (IHC) staining for arginase 1 will show a broad and intense distribution throughout the liver tissue. Immunohistochemistry is a technique used to detect arginase 1 in tissue samples. This technique uses specific antibodies that bind to arginase 1, which is then visualized through staining. In IHC, arginase 1 is often stained using monoclonal or polyclonal antibodies that impart a specific color (usually brown or red) at the location of the enzyme in the tissue. Arginase 1 is used as a marker to diagnose cellular hepatocarcinoma (HCC), a liver cancer. High expression of arginase 1 in a liver biopsy can help distinguish HCC from liver metastases or other liver tumors. Other histological staining techniques such as H&E (Hematoxylin and Eosin) staining may not specifically demonstrate arginase 1, although they can be used in conjunction with IHC to provide a general picture of tissue morphology and structure.

In rats treated with DMBA and rosemary 800 mg/kg BW with arginase-1 expression, the effect of the combination treatment was accompanied by an H-score value and there was a significant difference with other groups. In other samples, no arginase-1 expression was found and there was no significant difference between the normal control group, negative control, rats treated with DMBA with rosemary 200 mg/kg BW and rosemary 400 mg/kg BW. The phenomenon in this study explains that it can damage cell structure with 10 doses of DMBA and a longer exposure time is needed for the development of hepatocellular carcinoma formation. The assessment by immunohistochemical test is still qualitative, so it requires quantitative testing such as ELISA for further research. Arginase-1 immunohistochemistry in this study cannot be concluded because of weaknesses in this study, namely the method of cutting the preparations only once so that it does not ensure the formation of cancer.

b. Effect of Rosemary Leaf Ethanol Extract on the Expression of Tumor Suppressor p53 of Hepatocellular Carcinoma in Male Wistar Rats Induced by p-Dimethylamino benzaldehyde (DMBA).

p53 is a key transcription factor that regulates genes involved in tumor suppression. Mutations in p53, which occur in about 50% of human cancers, contribute to tumor development. It controls numerous genes that influence processes like apoptosis, cell cycle arrest, and DNA repair. p53 also plays a vital role in anti-tumor immunity by regulating factors such as TRAIL, DR5, TLRs, Fas, PKR, ULBP1/2, CCL2, the T-cell inhibitory ligand PD-L1, pro-inflammatory cytokines, immune cell activation, and antigen presentation. Genetic alterations in p53 can facilitate immune evasion by affecting immune cell recruitment, cytokine production in the tumor microenvironment, and inflammatory signaling [21].

The tumor suppressor gene P53 is one of the most frequently inactivated tumor suppressors in human cancers. The function of p53 during cancer development has been associated with a variety of transcriptional and non-transcriptional activities that lead to tight control of cell proliferation, senescence, DNA repair, and cell death. However, evidence suggests that the tumor suppressor p53 also plays a major role in normal and cancer cell metabolism [22].

Cancer cells often show various abnormalities or damage to the nucleus, cytoplasmic organelles, and cytoskeleton. The nucleus tends to be enlarged and irregularly shaped, with chromosome breaks, deletions, and translocations. The mitotic rate is usually increased. In the cytoplasm, intracellular structures appear disorganized and change in size and shape. Changes in microtubules, which support the cell and are essential for controlling nearly all cellular functions, have a major impact. Mitochondria also become disorganized and irregularly shaped [23].

P53 causes accumulation of various mutations. P53 functions to detect DNA damage and activate repair mechanisms. Genomic instability also causes increased epigenetic silencing or modulation of gene function. Increased DNA methylation in the promoter region of tumor suppressor genes is also found in many types of cancer. Increased DNA methylation is also associated with changes in histone modifications in

chromatin and often correlates with DNA methylation. Changes in the promoter region of genes cause gene deletion or changes in gene expression [24].

In this study, the biomarker studied was Tumor suppressor p53. This biomarker analysis was conducted to understand the expression of tumor suppressor p53 and its relationship to the development of hepatocellular carcinoma (HCC). The results of immunohistochemistry tests in this study showed the accumulation of tumor suppressor p53 in the cytoplasm, not entering the nucleus of liver tissue in rats that received rosemary leaf ethanol extract. These results indicate that tumor suppressor p53 is inactive. The results of other liver tissue immunohistochemistry tests showed the presence of active tumor suppressor p53 (brown), where the location of this active biomarker was at the edge of the tumor cell diameter of the liver tissue sample. Liver tissue in hepatocellular carcinoma model rats in this study showed abnormal cell shapes. During development, normal cells will differentiate. Differentiation means that a cell becomes specialized in its structure and function, and gathers with similarly differentiated cells. The more specialized a cell is, the higher the differentiation, the less often the cell enters the cell cycle and reproduces and divides. Cells that do not reproduce are highly differentiated cells.

The results of the immunohistochemical test of this study showed that the expression of tumor suppressor p53 differed between the normal group and the group treated with DMBA and rosemary ethanol extract. This study showed that Rosemary ethanol extract could inhibit the decrease in tumor suppressor p53 expression significantly in the group treated with DMBA, with an increase seen at doses of 400 mg/kg BW and 800 mg/kg BW. Rosemary leaf ethanol extract can inhibit the decrease in tumor suppressor p53 expression because it has strong overall antioxidant activity.

4. CONCLUSION

The ethanol extract of rosemary leaves demonstrated a potential to inhibit the decrease in Tumor Suppressor p53 expression but did not significantly affect the expression of Arginase-1 in hepatocellular carcinoma in male Wistar rats induced by p-Dimethylamino Benzaldehyde (DMBA).The effect of 800 mg/kg BW of rosemary leaf ethanol extract could not inhibit the increase in arginase-1 expression of hepatocellular carcinoma in male Wistar rats induced by p-Dimethylamino benzaldehyde (DMBA). Ethanol extract of rosemary leaves 400 mg/kg BW and 800 mg/kg BW inhibited the decrease in the expression of the tumor suppressor p53 from hepatocellular carcinoma in male Wistar rats induced by p-Dimethylamino benzaldehyde (DMBA).

DISCLAIMER(ARTIFICIALINTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ETHICAL APPROVAL

This study is a true experimental laboratories in vivo. This research protocol has met the ethical principles of research, the research protocol was reviewed and approved by the Udayana University ethics committee with a letter or Ethical Clearance document Number: B / 259 / UN14.2.9 / PT.01.04 / 2023. This research protocol has received approval for the implementation of research from the Integrated Biomedical Laboratory with a letter or document Number: 1568 / UN14.2.2.VII.6 / LT / 2023.

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