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ARTICLE

THE EFFECT HONEY HAS ON SOD AND CASPASE9 LEVELS WITH EXPOSURE TO CIGARETTE SMOKE IN WISTAR RATS

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ABSTRAK

Cigarettes are a source of exogenous free radicals which disrupt the balance between free radicals and *antioxidants* in the body, causing *oxidative stress.* The aim of this study was to determine the effect of giving honey (*Mel depuratum*) on *SOD* and *caspase-9* levels in Wistar rats exposed to cigarette smoke. This research used a laboratory experimental approach with *a post-test only control group design.* There are 24 Wistar rats were divided randomly into 4 groups then analysis tests were carried out using *the One-Way ANOVA* test and followed by *the Post-Hoc LSD* test. The results showed that the mean *SOD* levels in groups K1, K2, K3, K4 were 73.51 ± 1.68, 21.72 ± 2.23, 52.08 ± 2.03, and 73.51 ± 1.68. The results of *the One Way Anov*a test showed a significant difference in *SOD* levels with a value of p = 0.000. The results of *the LSD Post-Hoc* Test showed that *SOD* levels between groups had significant differences in all groups (p=0.000). *The IHK* staining results showed that samples K1 and K4 were negative, while K2 showed strong positive and K3 was moderately positive.

Keywords: Cigarette smoke ; Caspase9; Honey; SOD

АБСТРАКТ

Сигареты являются источником экзогенных свободных радикалов, которые нарушают баланс между свободными радикалами и *антиоксидантами* в организме, вызывая *окислительный стресс.* Целью данного исследования было определить влияние дачи меда (*Mel depuratum*) на уровни *СОД* и *каспазы-9* у крыс Вистар, подвергшихся воздействию сигаретного дыма. В данном исследовании использовался лабораторный экспериментальный подход с *контрольной группой после тестирования.* 24 крысы Вистар были случайным образом разделены на 4 группы, затем были проведены анализы с использованием *одностороннего* теста *ANOVA* и последующего теста *Post-Hoc LSD*. Результаты показали, что средние уровни *СОД* в группах K1, K2, K3, K4 составили 73,51 ± 1,68, 21,72 ± 2,23, 52,08 ± 2,03 и 73,51 ± 1,68. Результаты теста *One Way Anov*a показали значительную разницу в уровнях *СОД* со значением p = 0.000. Результаты *пост-хок* теста *LSD* показали, что уровни *СОД* между группами имели значительные различия во всех группах (p = 0,000). Результаты окрашивания*ИНК* показали, что образцы K1 и K4 были отрицательными, в то время как K2 был сильно положительным, а K3 - умеренно положительным.

Ключевые слова: Сигаретный дым ; Каспаза9; Мед; СОД

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INTRODUCTION

Cigarette smoke is a free radical which has molecules with one or more unpaired electrons in their outer orbit, which are very unstable and reactive. Free radicals will react with surrounding cell molecules to obtain electron pairs so they become more stable, but body cell molecules whose electrons are taken away will turn into free radicals. This reaction will continue continuously in the body and if it is not stopped it will cause *oxidative stress* which causes inflammation, *DNA* or cell damage, and various diseases such as *cancer, heart disease, cataracts, premature aging,* and other *degenerative diseases*.[1]

Oxidative stress causes various abnormalities in blood vessels. *Oxidative stress* is a condition where there is an excess of *reactive oxygen species* (*ROS*), so that it exceeds the ability of endogenous antioxidants in the body to neutralize *ROS*.[2] Endogenous antioxidants in the body include *SOD*, *catalase (CAT), glutathione peroxidase (GPx)*. *SOD* is one of the main endogenous antioxidant enzymes that is important in dealing with increased *ROS*. *SOD* has cell defense mechanisms both *endogenous* and *exogenous*. *SOD* also has an important role in reducing *atherosclerosis* due to *oxidative stress.*

Oxidative stress in the body can be overcome by using external antioxidants such as honey. Honey contains *flavonoid compounds, beta carotene*, vitamins A, B1, B2, B3, B5, B6, C, D, E, K, *phenolic acids, uric acid* and the minerals Fe, S, Mg, P, Cl which can act as antioxidants. Honey has a high antioxidant content, such as vitamins C, E, *beta-carotene*, and *flavonoid compounds,* which have the ability to reduce cell *apoptosis*.[3] *Apoptosis* will not occur without *caspase* activation. The involvement of *caspases* as marker indicators has potential for drug research. This form of *caspase-9* is an important marker for the entry point of the apoptotic signaling pathway in cells, where activation of this *caspas*e indicates cell damage.[4,5] Therefore, there is an urgency for immediate research to determine measurements of honey's effectiveness. Giving honey at a certain dose was the aim of this study to determine the levels of *SOD* and *Caspase9* in Wistar rats exposed to cigarette smoke.

MATERIAL AND METHODS

Research Population :The population for this study was male Wistar rats aged 10-12 weeks and weighing 200 grams. Wistar rats are maintained with standard comfeed AD II food and drinking water in the form of plain water with a rearing room temperature ranging from 28°-32° C and adequate ventilation and space. Then the Wistar rats were adapted for 7 days before being given treatment.

Sampling technique : The sample was obtained by allocating groups based on random sampling allocation. Twenty-four male Wistar rats that met the inclusion criteria were divided into 4 groups in simple random fashion with one control group and four treatment groups.

Examination of *Superoxide Dismutase (SOD)* levels is carried out using blood samples taken from the orbital sinus of the eye. Eye blood is taken using a *microhematocrit,* and the blood is collected in a microtube. *SOD* activity was measured using the *ELISA* method with *SOD* following the method developed by Asti.[6] The reduction of *ferricytochrome c* was observed by measuring the increase in absorbance at a wavelength of 550 nm. Changes in absorbance were observed using a *spectrophotometer*. Lung tissue samples were taken from Wistar rats 24 hours after the last treatment. The Wistar rats were then euthanized by cervical dislocation and encrypted according to standard procedures for harvesting lung organs.

The process of making paraffin blocks begins with dehydration, where tissue pieces are gradually soaked in graded alcohol, ranging from 30% to 96%, to remove fluid from within the tissue. Finally, the tissue attached to the slide is deparaffinized in an incubator by heating at a temperature of 56-58˚C until the paraffin melts. The *Caspase-9 Immunohistochemical* staining procedure

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follows standard methods from the UGM Food and Nutrition Study Center Laboratory. *Caspase-9* expression, marked in brown, was observed in cells using a light microscope at 400x magnification.

Statistical Analysis : Data analysis using the SPSS version 23 for Windows application. In this study, the average data on *SOD* levels is displayed and presented in descriptive and tabular form. Below, the data is tested for normality using the *Shapiro Wilk test* and the data *homogeneity test* using *the Levene test*. The distribution of data on SOD levels was normal and *homogeneous,* so it was continued with *a one-way ANOVA* parametric test (*One Way Anova*) with a p value <0.05, then continued with *a post hoc test.*

RESULT

SOD Level Measurement Results. Below is presented the data on the average results of *SOD* level examination:

Table 1. Data on the average results of *SOD* level examination

Variables	Group				P Value
	K i1 n i= i6 Mean±SD	K i2 n i= i6 Mean±SD	K i3 n i= i6 Mean±SD	K i4 n i= i6 Mean±SD	
Rate Isod					
Saphiro iWilk Levene iTest One iWay iAnova	80.06 i± i2.13 0.943*	21.72 i± i2.23 0.965*	52.08 i± i2.03 0.925*	73.51 i± i1.68 0.957*	0,0872# 0.000^

Note: * sign indicates normal data distribution results (p>0.05). The # sign indicates *homogeneous* data using the *Levene test* (p>0.05). The ^ sign indicates significant results for the *One Way Anova test* (p>0.05).

Source: Processed Primary Data (2023)

Based on the analysis results, it can be observed that the average *SOD* level in the control group (K1) was 80.06 ± 2.13. The treatment group without honey (K2) showed an average of 21.72 ± 2.23. Meanwhile, the group treated with honey at a dose of 0.8 ml/day (K3) showed an average of 52.08 ± 2.03, and the group treated with honey at a dose of 1.8 ml/day (K4) showed an average The average is 73.51 ± 1.68. Table 1 shows that the highest mean *SOD* level was in the control group, namely 80.06 ± 2.13, while the lowest mean value was in group 2 with a value of 21.72 ± 2.23. *SOD* levels between groups were normally distributed (p>0.05) and *homogeneous* (p>0.05). *SOD* levels between groups used *the one way anova test* with (p<0.05). The differences in *SOD* levels between the two groups showed that there were significant differences between K1 and K2, K3, K4. To find out which groups are different, *a post hoc test* was carried out. Next, the following table presents the results of the analysis of *SOD* levels using *the Post Hoc Test*:

Table 2. Results of Analysis of *SOD* Levels with *Post Hoc Test*

	K2	K3	K4
K1	0,000	0,000	0,000
K2		0,000	0,000
K3			0,000

Based on the results of the analysis, it was found that there was a significant difference between

each group in *SOD* levels (p<0.05). The difference is between K1 and K2 with a value of 0.000, K1 with K3 with a value of 0.000, K 1 and K4 with a value of 0.000. K2 with K3 with a value of 0.000, K2 with K4 with a value of 0.000, K3 with K4 with a value of 0.000. Based on the data above, it can be concluded that each group has significant differences in *SOD* levels.

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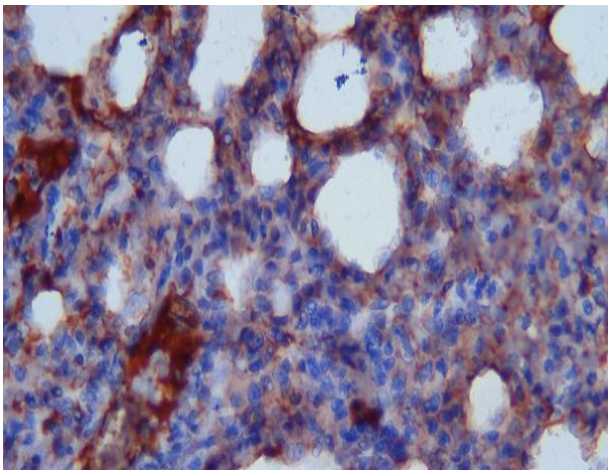
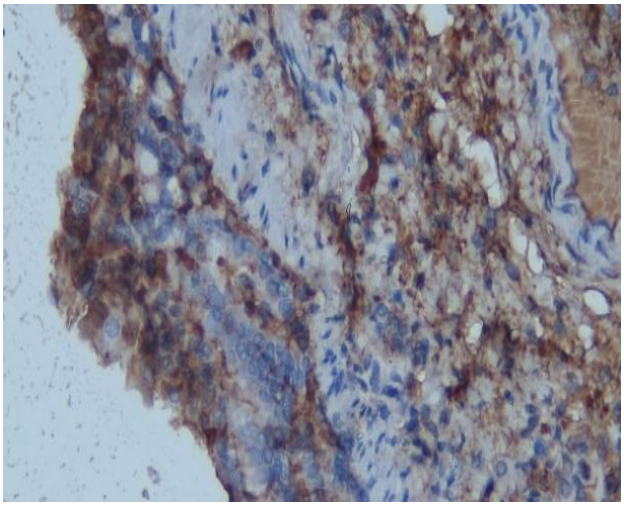
Results of *Caspase-9 Immunohistochemical* Examination in lung tissue

The results of observations of *Caspase-9 immunohistochemical* examination in lung tissue showed color changes in the *cell nuclei* and *cytoplasm* as the focus.

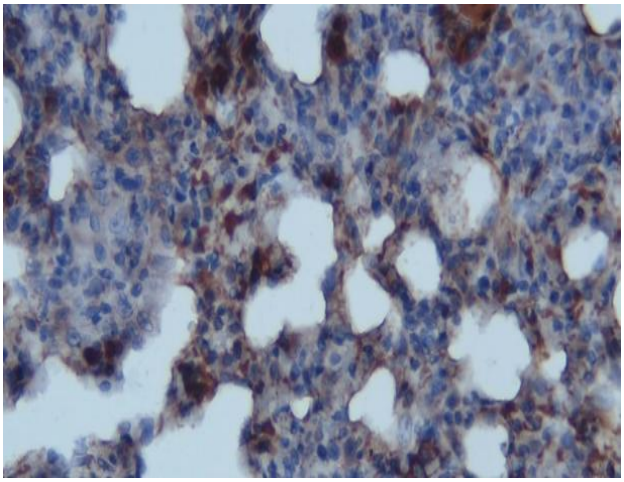
A.



B.



C.



D.

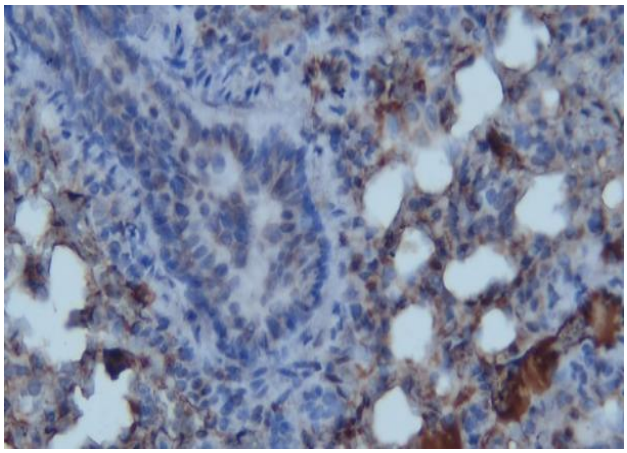


Figure 1. Shows the results of *immunohistochemistry* of lung organs with the following information: (A) Control group, (B) Treatment group with standard feeding which was exposed to cigarette smoke for 14 days; arrow indicates strong positive (K2), (C) Treatment group given honey at a dose of 0.9 ml/day who were exposed to cigarette smoke for 14 days; arrow indicates moderate positive (K3), (D) Treatment group given honey at a dose of 1.8 ml/day who were exposed to cigarette smoke for 14 days; the arrow indicates negative (K4). Image analysis shows that the control group without treatment (K1) showed negative results, while in the treatment group with standard feeding which received exposure to cigarette smoke for 14 days (K2), the interpreted results were strongly positive. Another treatment group, which received honey at a dose of 0.9 ml/day and was exposed to cigarette smoke for 14 days (K3), showed moderate positive results. Meanwhile, in the treatment group given honey at a dose of 1.8 ml/day and exposed to cigarette smoke for 14 days, the results showed a combination of positive, moderate and negative levels. These results provide an overview of the impact of exposure to cigarette smoke on *Caspase-9* activity in lung tissue, with specific explanations related to the effect of administering honey at various doses.

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DISCUSSION

SOD The results of the examination of levels in the group exposed to cigarette smoke without being given honey were lower compared to the control group, as seen in Table 1 which shows that exposure to cigarette smoke can reduce *SOD* levels. This is caused by exposure to cigarette smoke for 14 days which can cause *oxidative stress* conditions. When high *ROS* concentrations occur during prolonged exposure to cigarette smoke, *the SOD* antioxidant defense is unable to neutralize ROS, thereby causing cell and tissue damage.

SOD levels in the group given honey at a dose of 1.8 ml/200 grams BW and exposed to cigarette smoke were compared with the group given honey at a dose of 0.9 ml/200 grams BW and exposed to cigarette smoke. Even though *the SOD* level in the group with a dose of 1.8 ml/200 grams BW was the highest, it was still lower than the control group as shown in Table 1. This was because the control group was not exposed to cigarette smoke but still received standard feed so that *the SOD* level in the group it remains high.

Meanwhile, a comparison between the group given honey at a dose of 1.8 ml/200 grams BW and exposed to cigarette smoke and the group given honey at a dose of 0.9 ml/200 grams BW and exposed to cigarette smoke showed that the higher the dose of honey, the more also high levels of *SOD*. This shows that *the flavonoid*, Vitamin A and C content in honey can increase antioxidant *enzymes* and reduce peroxide levels. Previous research explains that giving honey can significantly increase the activity of *antioxidant enzymes*, such as *CAT, SOD, GST,* and *GPx*, compared to sick Wistar rats. This is caused by the antioxidant properties of honey which come from phenolic compounds. Phenolic compounds, with hydroxyl groups connected to aromatic rings, act as hydrogen donors in scavenging free radicals. In addition, phenolic compounds can act as electron donors and reduce metal ions. Therefore, the phenolic content is considered the key antioxidant property of honey.[7]

The research results are in line with Widowati which shows that honey with *flavonoid compounds* has antioxidant properties, as seen from the increase in *SOD* levels.[8] The research results are also in line with Parwata's research, which states that honey acts as an anti-free radical and increases *SOD* levels in the body. Parwata researched the effect of longan honey on *SOD*, while the results of this study highlight the effect of exposure to free radicals on *SOD* levels.[9]

This research is in line with Primayanti's research, which showed that exposure to cigarette smoke resulted in a decrease in the average serum *SOD* in the intervention group compared to the control group. The results of previous research also show that administration of honey can increase the activity of *antioxidant enzymes*, including *CAT, SOD, GST*, and *GPx*, which is due to the antioxidant properties of honey and *the phenolic compound* content. [10]

The decrease in *SOD* levels occurs due to *oxidative stress*, where the amount of free radicals is not balanced with the amount of *endogenous antioxidants*. Free radicals can be produced from various processes such as *oxidation, cell burning*, *excessive physical activity*, inflammation, and exposure to external pollution such as cigarette smoke. In conditions of *oxidative stress*, the activity of *SOD* as an *enzymatic antioxidant* can decrease, increasing the risk of cell damage and degenerative diseases. The importance of *SOD's* role as an antioxidant enzyme is also illustrated in the explanation of *oxidative stress* states. This research provides a further understanding of the balance between free radicals and antioxidants in the body, especially in the context of exposure to cigarette smoke. The presence of *endogenous antioxidants*, such as *SOD*, helps to neutralize free radicals, but in conditions of *oxidative stress, SOD* concentrations can decrease, increasing the body's susceptibility to cell damage and degenerative diseases.

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Free radicals in the body can be reduced by *endogenous antioxidants*, such as *SOD.* For example, the condition *hypercholesterolemia*, which is characterized by an increase in blood *cholesterol* levels, can increase the production of free radicals. This can trigger *oxidative stress*, which means the balance between free radicals and *endogenous antioxidants* becomes unstable. The level of *oxidative stress* can be measured by assessing *SOD* activity in blood. *SOD*, *an antioxidant enzyme* and *metalloenzyme*, depends on metal cofactors such as *Cu, Fe, Z*n, and *Mn*. Its function includes catalysis of the radical reduction of *soperoxide ion (O2*)* to *hydrogen peroxide (H2O2)* and *oxygen (O2).* Although this *enzyme* is unstable to heat, it is quite stable under *alkaline* conditions (PH 6.5-7.5), and remains active when stored for up to 5 years at a temperature of 5°C. The body has special compounds called *endogenous antioxidants* to fight free radicals. One of the main *endogenous antioxidants* that fights free radicals in the body is the *enzyme superoxide dismutase (SOD).* In conditions of *oxidative stress*, it is estimated that *SOD* concentrations will experience a decrease.[11,10,12]

Conditions of *oxidative stress* can be treated by providing external supplements or herbs, such as honey. Honey has been proven to have compounds such as *polyphenols* which can provide an antioxidant response by activating *AMPK (5'adenosine immunophosphate-activated protein kinase)* and *antioxidant enzymes* which can reduce *oxidative stress* conditions. Honey can increase the activity of *antioxidant enzymes* such as *SOD, catalase,* and *GPx,* so that *oxidative stress* in the mouse's body is reduced. Therefore, giving honey in research can influence the condition of Wistar rats exposed to cigarette smoke for 14 days.[10,12]

CASPASE-9

Group K1, which was not exposed to free radicals (cigarette smoke), showed negative *caspase-9 immunohistochemical* staining results, indicating the absence of cell damage. K2, which was exposed to free radicals without a honey dose, showed positive results for *caspase-9,* indicating cell damage due to exposure to free radicals. In K3, who were exposed to free radicals with a honey dose of 0.9 ml, the *caspase-9* staining results were strongly positive to moderately positive, indicating the presence of cell damage that could still be suppressed by administering honey with *a flavonoid* content of a dose of 0.9 ml. Furthermore, in K4, *the caspase-9* staining results ranged from moderately positive to negative, proving that the addition of a honey dose of 1.9 ml can suppress the occurrence of cell damage in the body.

The results of this research are in line with Porza's research on model Wistar rats that were given honey orally, increasing *SOD* levels as an indication of reducing *oxidative stress*. In addition, in Wistar rats given honey, there was a decrease in the expression of *pro-apoptotic proteins* such as Apaf-1 and *caspase-9*, as well as an increase in *anti-apoptotic proteins* such as *Cox-2* and *Bcl.*[13]

The results of this research are in line with Ahmed's research showing that honey has the ability to increase the amount and activity of *antioxidant agents* in the body as well as anticancer effects through interference with *the apoptosis pathway.*[14] Previous research also revealed that honey contains *polyphenols,* the main class of natural organic compounds determined by *the multiple of phenol units*. A number of *flavonoids,* such *as quercetin, myricetin, kaempferol, luteolin, routine, naringenin, naringin, chrysin, rhamnetin, isorhamnetin, apigenin, pinocembrin, pinobanksin, galangin, tricetin, catechin,* and *hesperidin,* are the main compounds found in various varieties of honey. This content makes honey potential as an *anti-apoptotic agent* and *antioxidant.*[15]

Cigarette smoke contains various chemical substances, including *oxygen* species and *reactive nitrogen (ROS* and *RNS)*, which can damage cellular and subcellular targets such as *lipids, proteins*, and *nucleic acids*. Increasing evidence supports the important role of *ROS* produced by smoking and *oxidative stress* resulting in *inflammation* and

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carcinogenesis.[16] Previous research shows that *oxidative stress* plays an important role in *apoptosis* in patients and models of *chronic obstructive pulmonary disease (COPD).* Smoking, as one of the risk factors for *COPD*, shows that smoking cessation appears to be the biggest risk factor.[16,17] *Apoptosis* is a cell death condition that occurs programmed, well regulated, and involves two main pathways, namely the extrinsic pathway or death receptors, and the intrinsic pathway or *mitochondria*.[18]

The intrinsic *apoptosis* pathway is a natural process that leads cells towards programmed cell death. The intrinsic apoptotic pathway activates a cascade of cell signals that is an integral part of the development and function of an organism. The intrinsic pathway of *apoptosis* is initiated when internal injury occurs in the cells and the resulting stress activates the pathway of *apoptosis*. In both intrinsic and extrinsic apoptotic pathways, signaling results in the activation of a family of *Cys (cysteine) proteases, called caspases,* which act in a *proteolytic cascade* to dismantle and eliminate dead cells.[19] The intrinsic apoptotic pathway begins when an internal injury occurs in the cell. Intrinsic stresses such as oncogenes, direct *DNA* damage, *hypoxia*, and deficiencies in survival factors, can activate intrinsic apoptotic pathways.Other *proteins* released from damaged *mitochondria,* such as *SMAC (Second Mitochondrial-Derived Activator of Caspase), Diablo, Arts,* and *Omi/HTRA2 (High Temperature Requirement Protein-A2),* counteract the effects of *IAP (Inhibitor of Apoptosis Proteins)*, which are usually binds and prevents activation of *Caspase-3.* Interactions between *IAPs* and members of *the Bcl family, SMAC*, and *Omi/HTRA2* are central to the intrinsic apoptotic pathway. Recent studies have shown that another *nuclease, EndoG (Endonuclease-G),* is specifically activated by apoptotic stimuli and is able to induce *nucleosome DNA* fragmentation independently of *Caspase* and *DFF (DNA-Fragmentation Factor)/CAD (Caspase-Activated DNAse). EndoG* is a *mitochondria-specific nuclease* that translocates to *the nucleus* and *cleaves chromatin DNA* during *apoptosis*. Another *protein, AIF (Apoptosis Inducing Factor),* also thought to play a role in *apoptosis*, becomes active after translocation from *mitochondria* to *nuclei,* where it initiates *chromatin condensation* and large-scale *DNA* fragmentation. [20]

From this process, the activated *Caspase-8* activates *Caspase-3* through two pathways. In the first pathway *Caspase-8 cuts BID (Bcl2 Interacting Protein)*, and its *COOH* terminal part translocates to the *mitochondria* where it triggers the release of *CytoC (Cytochrome-C).* The released *CytoC* binds to *APAF1 (Apoptotic Protease Activating Factor-1)* together with *dATP* and *Procaspase-9* and activates *Caspase-9. Caspase-9* cuts *Procaspase-3* and activates *Caspase-3.* Another pathway is that *Caspase-8* cuts *Procaspase-3* directly and activates it. *Caspase-3* cuts *the DNA* fragmentation factor *ICAD (Inhibitor of Caspase-Activated DNase*) in *a heterodimeric* form consisting of cleaved *CAD* and *ICAD*. The fragmented *ICAD* dissociates from *CAD*, inducing *oligomerization* of *CAD* which has *DNase* activity. Active *CAD oligomers* cause *internucleosomal DNA* fragmentation, which is a sign of *apoptosis* and indicates *chromatin* condensation.[21]

Apoptosis triggered by stress occurs through a mechanism that involves changes in *mitochondrial* permeability and the subsequent release of *CytoC* and the formation of *apoptosomes, a catalytic multiprotein* platform that activates *Caspase-9*. The activated *Caspase-9* then cleaves *Caspase-3*, resulting in downstream events that cause cell death. *CytoC* release is regulated by *Bcl2* family *proteins. Bcl2L (Bcl2-Like), BclXL (Bcl2 Related Protein Long Isoform),* and other members of the *anti-apoptotic Bcl2* family are located in the outer membrane of *mitochondria* and prevent the release of *CytoC. BAX (Bcl2 Associated x-protein), BID (BH3 Interacting Death Domain),* and *BIM (Bcl2-Interacting Protein)*

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are initially inactive and must be translocated to *mitochondria* to induce *apoptosis*, either by forming pores in *mitochondria* directly or by binding via *the BH3* domain to *Bcl2, BclXL*, and *Bfl1,* and antagonize these *anti-apoptotic proteins.*[22,23]

Caspases are a family of *cysteine-aspartic proteases* that mediate cell death and programmed inflammation. Cells express *caspase* as an *inactive enzyme*, which is rapidly activated in response to cellular signals. Initiator *caspases* (such as *caspase-1, -2, -8, -9, -10)* are activated via dimerization after binding to the activating platform. Once active, the initiator *caspase* activates downstream effector *caspases (such as caspase-3, -6,* and *-7)* through *proteolytic cleavage,* which in turn can activate additional initiator *caspases*.[24]

Caspase-9, an cysteine-aspartic protease, plays a crucial role as an intrinsic initiator of *apoptosis*, regulating physiological cell death and pathological tissue generation. In addition, *caspase-9* has functions outside of *apoptosis*, such as in the regulation of cell differentiation/maturation, the innate immune system, *mitochondrial homeostasis,* and *autophagy*. The form of *caspase-9* is an important marker at the cell entry point and the apoptotic signaling pathway, and activation of *caspases* indicates cell damage.[25] Previous research showed that exposure to cigarette smoke can increase the expression of *the caspase-9* gene, causing an increase in the mechanism of *apoptosis.* However, cell damage caused by *apoptosis* can be inhibited or reduced by the use of external antioxidants. The process of *apoptosis* is often associated with *oxidative stress;* Therefore, providing external antioxidants, such as honey, is expected to influence the process of *apoptosis*.[15,26]

The results of the study showed that the treatment group, which received honey at an dosage of 0.9 ml/day during exposure to cigarette smoke for 14 days, did not show a significant difference in *caspase-9 immunohistochemistry* compared to the control group who were given standard food during the same period. Based on this research, *the flavonoids* in honey are believed to have the ability to inhibit *the apoptosis* process by inhibiting *caspase-3* activity. *Flavonoids* have been proven to be key elements in signal transduction pathways such as *apoptosis*, and can trigger *apoptosis* through *immodulation* of a number of key elements in cellular signal transduction pathways associated with *apoptosis*. Previous studies have also shown that *flavonoids* can activate regulatory activities in cells through actions on various signal transduction pathways, including *caspases.*[15]

The research carried out by Abedi et. al. It is stated that honey has potential as *an antipoptosis* by modulating cellular signaling pathways such as *MAPK, NF-κB,* and *Nrf2.* Honey can also reduce inflammation, suppress the release of *cytokines,* and regulate *oxidative stress* and *apoptosis* through modulating various pathways such as *TNF, NF-κB, PI3K/Akt, MAPK*, and *apoptosis* pathways. In this study of Wistar rats exposed to cigarette smoke, the group given honey showed no significant difference in *caspase-9* expression compared with the control group. This research provides a broader view regarding the potency of honey and its components, such as *polyphenols* and *flavonoids,* in regulating cellular signaling pathways, *oxidative stress, inflammation,* and *apoptosis*.[27]

Limitations in this research are that it was not carried out to measure the level of *ROS*, which is a factor causing *oxidative stress,* as well as not being able to determine the total antioxidant capacity. In addition, this study also did not investigate the *molecular* mechanisms of direct administration of honey on the expression of *caspase-3, Cyt-c,* and *caspase-8.*

CONCLUSION

Giving honey can increase *SOD* levels in Wistar rats with results of K1 (80.06 ± 2.13), K2 (21.72 ± 2.23), K3 (52.08 ± 2.03; dose 0.8ml/day); K4(73.51 ± 1.68; dose 1.8 ml/day), giving honey also affected *caspase9* in Wistar

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rats with K1 results showing negative results, K2 being strongly positive, K3 being moderately positive, K4 a combination of moderate and negative positive levels.

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DECLARATIONS

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