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Virgin Coconut Oil (VCO) Attenuates Hepatotoxicity Induced by Cigarette Smoke in Rats

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ABSTRACT

Researchers have connected the antioxidants in virgin coconut oil (VCO) to the growing health benefits; however, little is known about the hepatoprotective and antioxidant properties of VCO against oxidative stress and liver damage brought on by smoking cigarettes. The research examined the hepatoprotective and antioxidant properties of VCO against oxidative stress and liver damage. Male Wistar rats were split into 4 groups: Group 1 (the control group) was fed rat pellets; Group 2 (the control positive group) was fed the basic diet and exposed to cigarette smoke; Group 3 was fed the basic diet and 0,45 ml VCO; Group 4 was provided the basic diet and 0,9 ml VCO. Serum liver biomarker (SGPT and SGOT) assays were performed after 28 days of therapy. When compared to the control group, exposure to cigarette smoke caused a substantial increase in blood liver enzymes. Treatment with VCO significantly prevented an increase of SGPT and SGOT levels compared to control positive group P2. This study demonstrated that inhaling cigarette smoke damages the liver and established the hepatoprotective properties of VCO against cigarette smoke-induced liver damage via reducing oxidative stress.

Keywords: Virgin Coconut Oil; Cigarette Smoke; Strain male white rat Wistar; Serum Glutamic Pyruvic Transaminase; Serum Glutamic Oxaloacetic Transaminase

INTRODUCTION

Smoking is a menace to health worldwide. Worldwide, diseases linked to tobacco use claim the lives of almost 8 million people each year. Around 1.2 million of these fatalities are caused by non-smokers' exposure to secondhand smoke, and roughly 7 million of these are directly attributable to tobacco use.(Organization, 2022) In Indonesia, tobacco use is quite prevalent. There are 3.7 million adult female smokers and 60.8 million adult male smokers in the nation, and juvenile tobacco usage has been gradually increasing in recent years. (WHO, 2020)

More than 7000 different substances can be found in cigarette smoke, many of which are harmful to human health. The majority of tobacco's ingredients are produced when burning tobacco and paper however, some are added during production (such as ammonia) and some are found naturally in tobacco, such as nicotine (e.g., acrolein).(Hall, Ribisl and Brewer, 2014) Certain detrimental health effects have been associated with various elements. For instance, two of the most detrimental substances to respiratory health are acrolein and acetaldehyde, and arsenic and hydrogen cyanide are very dangerous to cardiovascular health. (Noar *et al.*, 2018)

The damaging effects of smoking on the liver have not previously gotten much attention. It is far less clear how directly cigarette smoke affects extra-pulmonary tissues like the liver.(Fouda *et al.*, 2021) Smoking cigarettes was not thought to raise one's risk of contracting a chronic liver disease such as non-alcoholic fatty liver disease (NAFLD) or alcohol-associated cirrhosis. Yet, a growing body of studies indicates that smoking worsens liver disease, including fibrosis and liver cancer, and speeds up its course.(Rutledge and Asgharpour, 2020) Smoking appears to harm the liver in three different ways: carcinogenic, immunologic, and toxin-related (direct and indirect).(El-Zayadi, 2006) The oxidative stress caused by cytotoxic chemicals, which activate stellate cells and cause fibrosis, is one of smoking's direct negative impacts. Smoking increases the proinflammatory cytokines interleukin IL-1, IL-6, IL-8, and tumor necrosis factor, which harms liver cells.(Rutledge and Asgharpour, 2020) Persistent cigarette smoking affects various immunological processes, including the innate and adaptive immune systems. Nicotine prevents lymphocyte

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differentiation and proliferation, which suppresses the production of antibodies.(Ma, Long and Chen, 2021)

Cigarette smoke triggers free radicals which form Reactive Oxygen Species (ROS). Reactive Oxygen Species (ROS) are produced by oxidative stress in hepatocytes via cytochrome P450 pathways, and this process has been linked to direct hepatotoxic and hepatocarcinogenic consequences. Increased levels of gamma-glutamyl transferase, aspartate aminotransferase, and ALT were linked to this mechanism.(Markevych *et al.*, 2013) Cigarette smoke exposure has been proven to produce intermediate compounds having the potential to be cytotoxic, although not being in direct contact. For instance, cigarette smoke contains a significant amount of nicotine, which the liver metabolizes predominantly. Moreover, once within the body, nicotine may oxidize into cotinine, which has a longer half-life. It has been demonstrated that this mechanism decreases hepatic glutathione (GSH) concentration and catalase activity, which increases cellular ROS generation and worsens oxidative liver damage.(Taylor *et al.*, 2019) Indirect oxidative stress in hepatocytes may be brought on by nicotine and/or its intermediates, increased carboxyhemoglobin, and decreased oxygen supply. Fouda et al. have shown that carbon monoxide poisoning, a phenomenon known as cigarette smoke-exposure increases carboxyhemoglobin content to >5% and caused hepatocellular damage.(Fouda *et al.*, 2021)

Related to this, to protect liver cells from damage, a natural source that is high in antioxidants is needed, Virgin Coconut Oil (VCO). Fresh coconut kernels are used to create VCO, a modified oil with a clear color, a nice aroma, and a little amount of free fatty acids. The concentration of VCO contains antioxidants such as phenolic compounds, vitamin C, and vitamin E as well as medium chain fatty acids (MCFAs) like capric acid, caproic acid, caprylic acid, and lauric acid. These antioxidants help to combat oxidative stress brought on by free radicals.(Yeap *et al.*, 2015)·(Dumancas *et al.*, 2016)

Several studies have pointed to the advantages of VCO in preventing harm to liver cells. Margata, et al. indicated in a prior study that VCO can considerably lower serum levels of SGOT and SGPT in dyslipidemic rats.(Margata *et al.*, 2018) VCO also exhibits hepatoprotective potential against liver damage brought on by the antibiotics cyclophosphamide(Nair *et al.*, 2016) and trimethoprim-sulfamethoxazole(Otuechere *et al.*, 2014). Data on the hepatoprotective effects of VCO against methotrexate (MTX)-induced liver damage were presented by Famurewa et al. in Wistar rats by preventing oxidative stress, lipid peroxidation, and enhancing antioxidant enzyme activity.(Famurewa *et al.*, 2017)

Yet, little is known regarding the antioxidant and hepatoprotective effects of VCO against oxidative stress and liver damage brought on by cigarette smoking. This is despite the fact that the potent natural antioxidants in VCO have been connected to expanding health advantages. At this time, it is unknown if consuming VCO will lessen or protect the liver from cigarette smoke. The current study's goal was to determine whether VCO could protect rats' livers from damage caused by cigarette smoke by acting as an antioxidant and hepatoprotective.

METHODOLOGY

Materials

The DIALAB kits for serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase were utilized for biochemical tests of liver function parameters. The remaining reagents were all purchased and of analytical quality. The equipment used in the research is the smoking chamber, smoking pump (60cc syringe), digital balance, centrifugation, gastric sonde, and drink container. The Virgin Coconut Oil (VCO) used is VCO from brand Cocoseven, and the cigarettes used were kretek cigarettes from the Gudang Garam brand.

Male Wistar rats weighing 150–200 g were bought from a private animal breeding facility close to Sumatera Utara University. The rats were kept in standard environmental conditions and given unlimited access to pelleted commercial food mush and clean water. The rats had a week of acclimatization prior to treatment, and they were treated humanely by following established protocols for animal experiments. Ethical review for this study was obtain from Ethical Commitee from Faculty of Medicine, University of HKBP Nommensen, Medan (number : 376/KEPK/FK/VIII/2022).

Methods

A Post-Test Only Control Group design, which entails treating and studying the control group after it has received an action, was employed as the experimental strategy in this research.

After acclimation, the rats were split into 4 groups of 6 rats each, and the following meals were given to them: rats in Group 1 (the control group) were fed commercial rat pellets (the basic diet);

Rats in Group 2 (the control positive group) were fed the basic diet and exposed to cigarette smoke; Rats in Group 3 were fed the basic diet and 0,45 ml VCO; Rats in Group 4 were provided the basic diet and 0,9 ml VCO;

Other than the pipe holes, the rat cage for exposed cigarettes was created without any gaps. A dose of 4 cigarettes was administered once each day. A 60cc syringe is used by the modified smoking pump to simulate inhaling cigarette smoke. A pipe that is attached to a clove cigarette is attached to the syringe's end. The rats are then removed from their cages so they may breathe normally once the cigarettes are burned out and the smoke is pumped back into the smoking chamber.(David *et al.*, 2020) Four cigarettes were smoked each day for 28 days to expose subjects to cigarette smoke.(Sirait, Tjahjono and Setyawati, 2016) Virgin Coconut Oil (VCO) was given after exposure to cigarette smoke for 28 days in the treatment group. The Laurance table of 0.018 and the recommended dose of VCO for humans were used to calculate the administration of VCO with conversion units (25ml and 50ml).

Fasted animals were sacrificed at the conclusion of the treatment period (28 days in a row), and blood was obtained by heart puncture and centrifuged (3000 g for 15 min.) to separate the serum. Furthermore, the clotted blood cells were taken using a micropipette and then put into the Eppendorf tube. Then the SGOT and SGPT which had been mixed with the Kit reagent were measured using a spectrophotometer with a wavelength of 340 nm.

The software was used to carry out statistical analyses. Every value was expressed as the mean standard deviation of the mean. One-way analysis of variance (ANOVA) and Tamhane's Post Hoc Test were used to compare the evaluated parameters. Statistical significance was defined as a value of $p<0.05$.

RESULT AND DISCUSSION

Result

The biochemical indices of SGPT and SGOT were measured to evaluate liver function. Table 1 shows the effect of VCO supplemented on the mean level of SGPT and SGOT of Wistar rats.

As shown in table I the positive control group's SGPT and SGOT levels rise more when exposed to cigarette smoke (group P2). While the lowest SGPT levels were obtained in the group (P1) which was only given standard feed. SGPT and SGOT levels in group P2 showed a significant increase compared to the control negative group P1 (table 2). This demonstrates that liver function abnormalities were produced by group P2, which was only exposed to cigarette smoke.

On the other hand, in rats treated with VCO (group P3 and P4) SGPT and SGOT levels were lower. The supplemented diet with 0,45ml and 0,9ml VCO in groups P3 and P4 significantly prevented an increase of SGPT and SGOT levels compared to control positive group P2 ($p < 0.05$) as shown in table 2. Research demonstrates that administering VCO to rats exposed to cigarette smoke can prevent a rise in SGPT and SGOT levels. And in this investigation, a dose of 0.9 ml of VCO was the most effective at avoiding abnormalities of liver function. When compared to the P3 group, the SGPT and SGOT levels in the P4 group significantly decreased ($p<0.05$).

Discussion

Cigarette smoking, which is still prevalent in a sizable portion of the population, is one such unhealthy lifestyle component. It has long been known that smoking causes a stable free radical population to exist. These oxidants give tobacco smoke the ability to oxidize proteins, lipids, and DNA to harm organs, which may lead to hepatocyte damage, inflammation, or even fibrosis.(El-Zayadi, 2006)·(Markevych *et al.*, 2013) While they are regarded as safer, more accessible, and compatible with a regular diet, dietary treatments to reduce the hepatotoxicity of cigarette smoke may be preferable to pharmacological strategies. They also increase the safety of the medicine. Plant phenolic compounds are well known to be strong antioxidants and ROS scavengers. Due to their antioxidant, processed plant oils are receiving growing attention from the general population and scientific community.(Irawan *et al.*, 2022) We looked at whether VCO, which has strong antioxidant qualities, might protect rats' livers from damage and oxidative stress brought on by cigarette smoke.

The findings of the current study indicate that smoking has the potential to cause oxidative liver injury. The obvious changes in serum levels of liver damage indicators demonstrated this (SGPT and SGOT levels). High levels of SGPT and SGOT are known to be caused by acute liver damage. Since they are located in the cytoplasm and are released into circulation following hepatocellular damage, serum SGPT, and SGOT are the most sensitive indicators of liver impairment.(Agrawal and Gupta, 2013) SGPT and SGOT levels in group P2 showed a significant increase compared to the control negative group P1 (table 2). Each puff of cigarette smoke is thought to contain 10^{14} free radicals, which has been shown to be a significant source of

Table I. One Way Anova Results

Group		One Way Anova	
		Mean ± SD (μ/L)	***(p)***
SGPT	P1	100,5 μ/L ± 9,9	0,001
	P2	287,5 μ/L ± 52,9	
	P3	181,7 μ/L ± 18,4	
	P4	149,5 μ/L ± 34,0	
SGOT	P1	104,2 μ/L ± 13,1	0,001
	P2	281 μ/L ± 30,3	
	P3	175,3 μ/L ± 12,8	
	P4	150,2 μ/L ± 9,1	

Table II. Results of Tamhane's Post Hoc Analysis

Comparison of Treatment Groups							
SGPT	**P2**	**P3**	**P4**	**SGOT**	**P2**	**P3**	**P4**
P1	0,031*	0,001*	0,88	**P1**	0,003*	0,001*	0,001*
P2		0,133	0,040*	**P2**		0,021*	0,014*
P3			0,383	**P3**			0,021*

P1 = negative control group that was given standard feed; P2 = positive control group exposed to cigarette smoke and standard feed; P3 = The treatment group was exposed to cigarette smoke and given 0.45 ml of VCO; P4 = The treatment group was exposed to cigarette smoke and given 0.9 ml of VCO; * = Significant mean difference at 0.05

oxidant molecules and has been linked to the development of smoke-related lung diseases such as COPD. (Sumida *et al.*, 2013) It is far less clear how directly cigarette smoke affects extra-pulmonary tissues like the liver.(Fouda *et al.*, 2021)

According to research by Fouda et al, smoking causes liver inflammation. Exposure to cigarette smoke results in mild oxidative damage in the liver of normal mice.(Fouda *et al.*, 2021) Exposure to cigarette smoke has been demonstrated to produce intermediate compounds having cytotoxic potential. For instance, cigarette smoke contains a significant amount of nicotine, which the liver metabolizes predominantly. Moreover, once within the body, nicotine may oxidize into cotinine, which has a longer half-life. It has been demonstrated that this process decreases hepatic GSH content and catalase activity, increases cellular ROS production by causing mitochondrial malfunction, and causes oxidative liver damage.(Taylor *et al.*, 2019) Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and their removal by cellular antioxidants like GPx and other nonenzymatic molecules like glutathione (GSH).(Pizzino *et al.*, 2017)

Virgin coconut oil (VCO) is the best and purest form of coconut oil. VCO is gaining a reputation as the world's most healthful oil.(Yeap *et al.*, 2015) Recent research has demonstrated the wide range of therapeutic qualities of VCO, including its antiviral, antibacterial, antifungal, anti-inflammatory, antidiabetic, anti-obesity, antiulcerogenic, analgesic, antipyretic, and antioxidant properties.(Dumancas *et al.*, 2016).

Several studies have pointed to the advantages of VCO in preventing harm to liver cells. VCO has the ability to protect the liver from harm caused by antibiotics, cyclophosphamide (Nair *et al.*, 2016), and trimethoprim-sulfamethoxazole(Otuechere *et al.*, 2014). Data on the hepatoprotective effects of VCO against methotrexate (MTX)-induced liver damage were also presented by Famurewa et al. in Wistar rats by preventing oxidative stress, lipid peroxidation, and enhancing antioxidant enzyme activity.(Famurewa *et al.*, 2017) These findings support earlier publications that found VCO reduced increases in SGPT and SGOT levels. This finding indicates that in rat livers exposed to cigarette smoke, polyphenols from VCO were able to preserve the enzyme activity. Protocatechuic, vanillic, caffeic, syringic, ferulic, and catechin are among the phenolic chemicals found in VCO. (Srivastava, Semwal and

Majumdar, 2016) Polyphenols' capacity to scavenge free radicals may help explain why VCO in this study had antioxidant and hepatoprotective effects. These findings support earlier research indicating that the antioxidant phenolic content of VCO may contribute to its positive health effects. (Nair *et al.*, 2016)· (Otuechere *et al.*, 2014)·(Arunima S, 2013)

CONCLUSION

In this regard, the current study has demonstrated that smoking induces liver damage and has established the hepatoprotective properties of VCO against smoking-induced hepatic damage via the regulation of oxidative stress. Bioactive polyphenols purportedly preserved in VCO may be the cause of the positive health benefit.

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