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Original Article

COMBINATION OF NIGELLA SATIVA AND CURCUMA XANTHORRHIZA ROXB. REDUCES HYPERLIPIDEMIA IN NEPHROTIC SYNDROME

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Abstract

Background: PNS (primary idiopathic nephrotic syndrome) is a frequent renal nephropathy that manifests as severe proteinuria and hypoalbuminemia. Some individuals have poor reactions and clinical results when treated with conventional medicine. Despite significant advancements in its treatment, there are still ongoing searches to seek treatment. *Curcuma xanthorrhixa* Roxb. (temulawak) and *Nigella sativa* (black cumin) rhizome contain many biological properties, including potent antioxidant and renoprotective effects.

Aim: This study sought to determine whether the combination of *Nigella sativa* and *Curcuma xanthorrhiza* Roxb. reduces hyperlipidemia in Nephrotic syndrome.

Methods: This was an experimental study with a posttest-only control group design involving 24 male Wistar rats divided into three groups; the negative control group received no treatment, the positive control group received an adriamycin (ADR)6,5 mg/kg BW injection, and the intervention group received rhizome extract temulawak (1000 mg/kg BW) and black cumin (250 mg/kg BW). The serum levels of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol are examined post-treatment.

Results: The combination of *Nigella sativa* extract and *Curcuma xanthorrhiza* rhizome reduced total cholesterol (p=0.006) and triglyceride (p=0.003). There was no significant result observed for HDL(p=0.432) and LDL-c (p=0.317) (Kruskal-Wallis test; p <0.05).

Conclusion: Temulawak and black cumin extracts can reduce total cholesterol and triglyceride. However, no significant result was observed in NS model rats in HDL and LDL levels.

Keywords: Nephrotic syndrome, lipid profile, *Curcuma xanthorrhiza* Roxb., and *Nigella sativa*.

INTRODUCTION

Nephrotic syndrome (NS) is one of the world's most prevalent juvenile renal illnesses, with a reported incidence of 2.1–16.9 per 100,000 children (Shatat et al., 2019). Corticosteroids are the mainstay of treatment for children with nephrotic syndrome, and steroid sensitivity is the most prevalent predictor of prognosis (Carter et al., 2020). When there is severe hypoalbuminemia, oedema, proteinuria, and maybe hyperlipidemia, nephrotic syndrome is acceptable. Children with this ailment frequently experience periorbital swelling in infancy, along with or without body oedema. When the Glomerular filtration barrier (GFB) experiences structural and functional problems, it becomes challenging to control the amount of protein in the urine, which leads to the onset of nephrotic syndrome (Vallepu et al., 2019). NS is characterized by hypertriglyceridemia and hypercholesterolemia due to increased apolipoprotein B-containing lipoproteins, decreased lipoprotein lipase and hepatic lipase activity, increased hepatic PCSK9 levels, and impaired hepatic uptake of high-density lipoprotein (Hari et al., 2020). The main factor contributing to the beginning and progression of numerous diseases is oxidative stress. Almost 80–90% of children with nephrotic syndrome react to steroid medication, which biopsy results could not predict (Sinjvens et al., 2021). Although the exact origin of idiopathic nephrotic syndrome is unknown, immunological dysregulation, systemic circulating substances, or hereditary structural abnormalities of the podocyte are thoughtto play a role in the condition's pathophysiology. In order to effectively treat podocyte injury, immunosuppressants in combination with podocyte-targeted therapy seem to be a better choice as the initial course of SRNS treatment (Zhao & Liu, 2020). There is a need to look for other kinds of medicine, like herbal plants. Temulawak (*Curcuma xantorrhiza* Roxb.) and black Cumin (*Nigella sativa*) are common herbal plants in Indonesia (Sumaryada & Pramudita, 2021). Because they contain antioxidant compounds, they could be used to treat NS. The phenolic OH compounds in curcuminoids from temulawak and black cumin can react with H atoms (Nova et al., 2021). Therefore, it can fight free radicals, possess an antioxidant effect, and protect the kidneys. The role of COX2 and iNOS genes by changing the way transcription factors bind to DNA (Hassan et al., 2019).

NS made oxidative stress worse and made antioxidants less effective. Black cumin (*Nigella sativa*) has an antioxidant compound called thymoquinone. The mechanism, which involves the antioxidant thymoquinone, controls redox systems that can catch free radicals (Hassan et al., 2019). For patients who do not respond as well to traditional medication, herbal medicine treatments for pediatric RNS can be given as a new option (Jang et al., 2020). In DOX-induced podocytes, curcumin activated Nrf2, blocked the NF-B pathway, and increased podocin.

Additional studies revealed that curcumin could significantly reduce proteinuria, enhance hypoalbuminemia in NS rats, and lower blood lipid levels to reduce hyperlipidemia (Fan et al., 2020). The primary factor in the genesis and progression of numerous diseases is oxidative stress. An essential antioxidant system that protects the redox equilibrium of cells is the Keap1-Nrf2 pathway. It lessens inflammation by encouraging diverse antioxidant responses in cells through the nuclear trans-localization of Nrf2 as a transcription factor (Ghareghomi et al., 2021). Although inflammation and oxidative stress are essential defensive mechanisms against infections, they can also have several harmful effects if improperly controlled, including the overproduction of cytokines and increased oxidative stress and inflammation (Rapa et al., 2020). Adriamycin (ADR) was used in this study to induce NS in mice. ADR belongs to a class of known anthracycline antibiotics and has a nephrotoxic effect, enabling it to be used in investigations to induce nephrotic syndrome in rats. ADR is thought to be primarily caused by the formation of free radicals (Varela-López et al., 2019). This study sought to determine the effects of black cumin (*Nigella sativa*) and temulawak rhizome extract (*Curcuma xanthorrhiza* Roxb.) against the lipid profile of adriamycin-induced nephrotic syndrome rats.

Recent research has shown that curcumin affects lipid metabolism, which may help prevent atherosclerosis and hyperlipidemia. A distinct, detrimental risk indicator of cardiovascular disease is plasma HDL cholesterol (HDL-C) (CVD). However, there have been deflating findings about the therapeutic advantage of increasing plasma HDL-C levels in several clinical and genetic research studies (Ganjali et al., 2017).

METHODS

Sample

The experimental animals employed in this study were healthy-appearing mice and male Wistar rats (*Rattus norvegicus*), which were eight weeks old before adaption and weighed 200–300 grams without anatomical/ structural deformity.

Extract preparation

The registered laboratory confirmed the herbal purchased with voucher no. SP 41/10/22. ***Curcuma xanthorrhixa* Roxb.** Rhizomes that have been well-cleansed, finely cut, and dried (without exposure to the sun). The ginger rhizome was ground using a blender, then 10 grams of ginger rhizome extract powder was weighed into an Erlenmeyer, and 100 ml of 95% ethanol solvent was added, or a sample-to-solvent ratio of 1:10 was achieved. The maceration was performed by shaking and stirring at 37°C. Three 24-hour periods of maceration were performed to create macerate. Using the filter paper, the macerate was filtered to generate filtrate, which was then concentrated using an 7% w/w EYELA rotary evaporator. In order to remove the remaining ethanol from the concentrated extract and produce a crude extract, it was incubated at 45°C for three days. ***Nigella sativa*** using a blender, black cumin is grounded to a fine powder. Ten grams of ginger rhizome extract powder were weighed, placed in an Erlenmeyer, and 100 milliliters of 96% ethanol were added, resulting in a sample-to-solvent ratio of 1:10 (or a sample-to-solvent volume ratio of 1:10). The Erlenmeyer was covered with aluminum foil before being macerated at 37ºC by shaking and stirring. The maceration procedure was conducted for 20 hours, and then the maceration was allowed to stand for 24 hours. The filtrate was concentrated at 45º C using an EYELA rotary evaporator (7% w/w) such that only black cumin extract remained.

Chemical and reagents

For reading, we used UV5Bio UV/Vis Spectroscopy for Life Sciences Spectrophotometer at the Center for Biomedical Research, Universitas Diponegoro. Life sciences instrument for UV/VIS based on a cuvette. The reagents used were Cholesterol FS 10, Triglycerides, HDL-Cholesterol, and LDL-c direct FS from Diasys Diagnostic System. In order to determine the total phenolic content (TPC) of phenolic compounds in the herbal, the Folin-Denis Spectrophotometry method was employed (Leonelli et al., 2012).

Laboratory testing. Cholesterol assay. Serum cholesterol levels were measured using a spectrophotometer and the cholesterol technique paraaminophenazone oxidase; diasys (CHOD-PAP). The blood sample was centrifuged for 10 minutes at 3000 rpm and 3000 rpm for 10 minutes. Using a micropipette, 5 mL of serum samples were transferred to the cuvette. 500 L of reagent kit (Diasys Cholesterol FS) was added using a micropipette. Twenty minutes at 20°C to 25°C or 10 minutes at 37°C for the incoming sample during incubation. The absorbance of total cholesterol is measured at a wavelength of 500 nm against a reagent blank of 5 L distilled water and a reagent kit of 500 L within 60 minutes. **Triglyceride measurement.** Enzymatic colorimetric glycerol peroxidase phosphate acid (GPO-PAP) and glycerol peroxidase phosphate acid (GPO-PAP) were used to determine the triglyceride levels in blood serum. Using a micropipette, 1 mL of triglyceride reagent was applied. Ten minutes at 20°C to 25°C or 5 minutes at 37°C for the incoming sample during incubation. The absorbance of triglycerides was evaluated using a wavelength of 546 nm, a reagent blank containing 10 L of distilled water, and a reagent kit containing 1,000 L of distilled water for sixty minutes. **HDL measurement.** Blood serum HDL levels were measured utilizing the spectrophotometer and the enzymatic cholesterol oxidase para aminophenazone; diasys (CHOD- PAP) method. The blood is centrifuged at a high speed of 3000 revolutions per minute for 15 minutes. Insert 20 mL of serum sample into the cuvette using a micropipette. 500 L of HDL reagent was added using a micropipette. Ten minutes of incubation at 20 to 25ºC or 37ºC. The absorbance of HDL cholesterol was determined using a wavelength of 546 nm, a blank reagent containing 5 L of distilled water, and a reagent kit containing 500 L of reagent within 60 minutes. **LDL-C measurement**. Blood serum LDL levels were determined using the spectrophotometer and enzymatic techniques cholesterol oxidase paraaminophenazone; diasys (CHOD-PAP). The blood sample was centrifuged for 15 minutes at 3000 rpm and 3000 revolutions per minute. Utilizing a micropipette, transfer 20 mL of a serum sample to the cuvette. 500 l of working reagent was added with a micropipette. Ten minutes of incubation at 20 to 25ºC or 10 minutes can be completed at 37ºC. LDL cholesterol

absorbance was measured using a 546 nm wavelength against a reagent blank comprising 5 L of distilled water and a 500 L reagent kit within 60 minutes.

Animal Treatment

All rats were kept in cages in the Animal Laboratory of the Faculty of Medicine at Diponegoro University. Given access to ad libitum traditional food and drink for seven days to facilitate adaptation, the rats consume 3 to 4 grams of dry food per day, or about 20% of their body weight, and require 3 milliliters of water per day. The standard animal feed consists of pellets having 20% protein. The rats were then placed into three groups randomlyon the eighth day following the adaption treatment. The negative control group consisted of untreated healthy rats, and the positive control group consisted of rats administered ADR 6.5 mg/kg BW. The treatment group consisted of rats administered ADR 6.5 mg/kg BW together with *Curcuma xanthorrhixa* Roxb. rhizome extract 1000 mg/kgBW and black cumin (*Nigella sativa*) extract 200 mg/kg BW.

Analytical Statistics

IBM SPSS Statistics Version 27 was used to complete all statistical calculations.

RESULTS

Before the study, we examined the containment of flavonoid compounds in *Curcuma xanthorrhixa* Roxb. and Black cumin (*Nigella sativa)* **(Table 1).** In this study, 24 Wistar rats (Rattus norvegicus) were employed, and the samples were then separated into three groups. Each group included eight rats. Each rat was administered a group treatment, and subsequently, serum LDL cholesterol, serum HDL cholesterol, total serum cholesterol, and serum triglycerides were measured. The collection of data was conducted two weeks after the intervention.

Table 1. Flavonoid compound in *Curcuma xanthorrhixa* Roxb. and Black cumin (*Nigella sativa)*

Sample	**µg QE/g**	**mg QE/g**	**%Flavonoid**
Curcuma xanthorrhixa Roxb.	432806	432,806	43,2806
	433715	433,715	43,3715
	433715	433,715	43,3715
Nigella sativa	827,4232	0,827	0,0827
	833,3333	0,833	0,0833
	827,4232	0,827	0,0827

Table 2. Serum cholesterol concentrations of Wistar rats in each group

Group	**Cholesterol (mg/dL)**		
	Mean ± SD	**Median (min-max)**	*p**
Negative control group	42 ± 10.53	49 (30 – 51)	
Positive control group	111.38 ± 90.88	74 (54 – 318)	
Treatment group	58.33 ± 19.33	55 (31 – 81)	0.006

Note: The negative control group consisted of untreated healthy rats, the positive control group consisted of rats administered ADR 6.5 mg/kg BW, and the treatment group consisted of rats administered ADR 6.5 mg/kg BW together with *Curcuma xanthorrhixa* Roxb. rhizome extract 1000 mg/kg BW and Black cumin (*Nigella sativa*) extract 200 mg/kg BW.* Kruskal–Wallis H test

The Kruskal Wallis test for serum cholesterol in the therapy group yielded a *p*-value = 0.006 **(Table 2)**, indicating a significant difference in serum cholesterol levels between the groups ($p<0.05$).

Table 3. Triglycerides level in each group of Wistar rats

Group	**Triglycerides level (mg/dL)**		
	Mean ± SD	**Median (min-max)**	***p****
Negative control group	42 ± 10.53	48 (36 – 178)	
Positive control group	111.38 ± 90.88	161.5 (91 – 468)	0.003
Treatment group	58.33 ± 19.33	131 (36 – 392)	

Note: The negative control group consisted of untreated healthy rats, the positive control group consisted of rats administered ADR 6.5 mg/kg BW, and the treatment group consisted of rats administered ADR 6.5 mg/kg BW together with *Curcuma xanthorrhixa* Roxb. rhizome extract 1000 mg/kg BW and black cumin (*Nigella sativa*) extract 200 mg/kg BW.* Kruskal–Wallis H test

Table 3. displays the results of the Way ANOVA test, with the Kruskal-Wallis test having a $p = 0.003$. Because the p-value for this analytical test was less than 0.05, the results demonstrate a significant difference in serum triglyceride levels between the groups.

Table 4. Results of the assessment of blood HDL levels in each group of Wistar rats

Group	**HDL (mg/dL)**		
	Mean ± SD	**Median (min-max)**	***p****
Negative control group	19.20 ± 4.60	21 (14 – 25)	0.432
Positive control group	38.13 ± 15.92	33 (25 – 75)	
Treatment group	24.17 ± 7.27	23 (14 – 35)	

Note: The negative control group consisted of untreated healthy rats, the positive control group consisted of rats administered ADR 6.5 mg/kg BW, and the treatment group consisted of rats administered ADR 6.5 mg/kg BW together with *Curcuma xanthorrhixa* Roxb**.** rhizome extract 1000 mg/kg BW and Black cumin (*Nigella sativa*) extract 200 mg/kg BW.* Kruskal–Wallis H test

Each treatment group's mean HDL serum levels are compared (**Table 4**), and the findings demonstrated no significant differences between the groups.

Table 5. Results of calculating LDL serum levels in each group of Wistar rats

Group	LDL (mg/dL)		
	Mean ± SD	Median (min-max)	*p**
Negative control group	9.20 ± 5.93	10 (2 – 18)	
Positive control group	35.13 ± 46.52	15 (10 – 145)	0.317
Treatment group	12 ± 6.06	12 (2 – 18)	

Note: The negative control group consisted of untreated healthy rats, the positive control group consisted of rats administered ADR 6.5 mg/kg BW, and the treatment group consisted of rats administered ADR 6.5 mg/kg BW together with *Curcuma xanthorrhixa* Roxb. rhizome extract 1000 mg/kg BW and Black cumin (*Nigella sativa*) extract 200 mg/kg BW.* Kruskal–Wallis H test

Table 5. shows a comparison of the average LDL serum levels for each treatment group. The Kruskal Wallis test for blood LDL in the treatment group indicated a *p*-value = 0.317. The findings of this analytic test showed no significant difference in serum LDL levels in any group.

DISCUSSION

It has been noted that the rhizome-derived phenolic component curcumin exhibits a wide spectrum of biological activity, including potent antioxidant and renoprotective effects. Curcumin may shield the kidneys, according to some research (Fan et al., 2020). Infiltration of macrophages in the kidney and proteinuria are both greatly decreased by curcumin, and the disease model is also improved (Ghosh et al., 2014). In a study, herbal treatments were shown to be superior to western medicine in treating PNS concerning overall clinical efficacy, 24-hour urinary protein excretion, and serum albumin, according to a study on Chinese Herbal Injections for the Treatment of Primary Nephrotic Syndrome. (Yu et al., 2020). Curcumin increases -tocopherol levels in rat plasma while decreasing TC levels in the liver, indicating an in vivo interaction between curcumin and tocopherol, which may boost Vitamin E's absorption and lower cholesterol levels. (Yu et al., 2020)

There have been numerous reviews demonstrating how curcumin can regulate a variety of enzymes, cytokines, transcription factors, growth factors, receptors, microRNA (miRNA), signaling molecules, and reactive oxygen species in order to reduce inflammation and protect against oxidative stress (Ghareghomi et al., 2021). Through the inhibition of phosphorylation and degradation of inhibitor of B inhibition of inhibitor of B-kinase activity, and inhibition of NF-B nuclear translocation, curcumin reduced IL-1-induced NF-B activation. Additionally, wortmannin reversed the effects of IL-1, showing that the phosphatidylinositol 3-kinase (PI-3K) pathway participates in IL-1 signaling. The IL-1-induced activation of PI-3K/p85/Akt and its interaction with IKK were inhibited by curcumin (Fan et al., 2020).

As a medication that can cause a nephrotic syndrome in rats, adriamycin (ADR) has long been well-known. ADR is a member of the anthracycline antibiotic class, which is recognized for its nephrotoxic effects (Mohajeri & Sahebkar, 2018). ADR nephrotoxicity is thought to be connected to the production of free radicals, oxidative damage to iron-related oxidants, membrane lipid peroxide (LPO), and the oxidation of proteins that cause tissue damage (Mohajeri & Sahebkar, 2018). An injection of adriamycin can stop the carnitine palmitoyltransferase (CPT1) system and lower the cytochrome P450. This will decrease the activity of the enzyme cholesterol-7-hydroxylase (CYP7A1), which helps break down cholesterol into bile acids. If CYP7A1 is turned down, the breakdown of cholesterol will slow down. This will cause total cholesterol levels to go down (Ossoli et al., 2022). Rats injected with adriamycin have lower levels of triglycerides because lipoprotein lipase activity is turned down. Decline Triglyceride breakdown slows down when lipoprotein lipase is active, which lowers the number of triglycerides in the blood. LDL levels in the blood go up because of a drug called adriamycin. This was established

by lowering LDL receptors, which play a role in breaking down LDL (Khetarpal et al., 2021). ADR results in an increase in total cholesterol levels and triglyceride levels. According to an earlier study, ADR injection can inhibit carnitine palmitoyltransferase (CPT1) system and lower cytochrome P450 levels. This mechanism will reduce the activity of cholesterol-7-hydroxylase (CYP7A1), an enzyme involved in converting cholesterol to bile acids. Reducing cholesterol catabolism via inhibition of CYP7A1 will decrease total cholesterol levels (Afsar et al., 2020).

Extracts of temulawak and black cumin help lower triglycerides and total cholesterol. However, there was no discernible difference in HDL and LDL values across model rats. Temulawak rhizome's anti-dyslipidemic effect is due to the curcumin in its curcuma rhizome. Curcumin can lower cholesterol levels by increasing the activity of the liver enzyme cholesterol-7-hydroxylase (CYP7A1) (Mauren & Lay, 2016). The CYP7A1 enzyme is an enzyme that helps break down cholesterol into bile acids. Therefore, a rise in the activity of the CYP7A1 enzyme will lead to a drop in total cholesterol. Curcumin can also cause a drop in cholesterol levels. LDL by upregulating LDL receptors. The number of LDL receptors begins with activating the peroxisome proliferator-enzyme activated receptor- (PPAR-). The liver's regulatory sterols element-binding protein-1 (SREBP-1) works to increase the number of LDL receptors (Rizzolo et al., 2021; Varghese et al., 2019). This transcription factor raises the number of LDL receptors. PPAR activation can also raise HDL cholesterol levels. PPAR activation raises HDL cholesterol levels by increasing the expression of Apo A-1 and Apo A-2 components (Varghese et al., 2019).

The curcumin containment in temulawak rhizome extract can alleviate dyslipidemia in patients with nephrotic syndrome. Encouraging the growth of hepatic stellate cells, the curcumin in temulawak rhizomes can lower LDL synthesis. Due to curcumin components' ability to induce the hepatic enzyme cholesterol-7-hydroxylase, ginger rhizome extract can lower LDL cholesterol and total cholesterol levels in individuals with nephrotic syndrome (CYP7A1) (Rahmat et al., 2021). CYP7A1 enzymes accelerate the conversion of cholesterol to bile salts. Increased CYP7A1 will cause an increase in cholesterol catabolism, resulting in decreased cholesterol levels and increased plasma LDL absorption by LDL receptors (Rahmat et al., 2021). Peroxisome proliferator-activated receptor (PPAR) activation is another action of curcumin. As an activator of sterol regulatory element-binding protein-1 (SRBP-1) in the liver, they increase LDL receptors and decrease LDL cholesterol (Rufino et al., 2021).

HDL is a particle of protein that will raise cholesterol levels. Curcumin can also interact with lipoprotein lipase (LPL) to increase the amount of LPL and the blood's ability to break down triglycerides (Rufino et al., 2021). Black cumin makes LDL, total cholesterol, and Triglyceride levels go down, increasing HDL levels. This happens because the compounds in black cumin, like thymoquinone and flavonoids, work together. These compounds help reduce the activity of HMGCoA reductase, increase the number of LDL receptors, stop cholesterol from forming, and are reactive oxygen species, which helps the kidney heal from oxidative stress (Rufino et al., 2021).

Thymoquinone compounds can increase the expression of the LDL receptor gene, which helps break down LDL. They can also stop the enzyme HMG Co-A-Reductase from working, which stops cholesterol from being made (Ojueromi et al., 2022; Rufino et al., 2021). Meanwhile, flavonoid compounds can help treat oxidative stress in the kidney caused by injecting adriamycin by stopping the body from making cholesterol and lowering the number of reactive oxygen species. Flavonoids can also make more LDL receptors, speeding up the breakdown of LDL cholesterol (Rufino et al., 2021; Wen et al., 2021).

Black cumin seeds are reported to contain the significant compound fixed oil (32-40%), essential oils (1%), fatty acids, oleic, linoleic, and p-symena acids, acids palmitate, fatty acids, tocopherols, sterols, and such alkaloid compounds as nigellimine and nigellidine—one of the substances that contributes to Thymoquionone accounts for most of the black cumin's pharmacological activity (Gawron et al., 2021). Black cumin essential oil contains the nonpolar chemical thymoquinone. It is well known that black cumin seeds have antibacterial properties. anticancer, anti-inflammatory, cytotoxic, antioxidant, and immunostimulant. They are operating PPAR, the peroxisome proliferator-activated receptor. Sterol regulatory element-binding protein-1 (SRBP-1) in the liver increases LDL receptors and decreases LDL cholesterol (Pelegrin et al., 2019).

Conclusion

Triglycerides and total cholesterol can be reduced with temulawak and black cumin extracts. However, the HDL and LDL values across NS model rats showed no appreciable variation. The precise molecular and cellular mechanisms behind the hypocholesterolemic characteristics of *Nigella sativa* and *Curcuma xanthorrhixa* Roxb., as well as the effects of its ingredients, require additional study. It was also necessary to conduct an additional clinical study on the hypocholesterolemic effects of the plant and its constituent parts.

Ethical clearance

KEPK has granted ethical clearance with the number 90/EC/H/FK-UNDIP/VIII/2022.

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Contribution of Author

Formal analysis, research execution by ANS and JRH data curation, ANS: Writing, including drafting the first draft, Writing, editing, and review for ANS and KT, the acquisition of funding, JRH. Methodology, KT, and ANS Validation of the ANS, RH, and PKD. Formal analyses FF, PKD, RH, ANS, inquiry, ANS, and JRH. The last draft has been read through by all authors and approved.

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