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AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 24 No. 9
September 2024
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2024; 24(9): 24448-24469 https://doi.org/10.18697/ajfand.134.25205

Date	Submitted	Accepted	Published
	5th August 2024	13th September 2024	4th October 2024

THE EFFECT OF POLYHERBAL CONTAINING *TAMARINDUS INDICA* AND *MURRAYA PANICULATA* LEAF EXTRACT ON TRIGLYCERIDE LEVEL USING A RAT MODEL OF HYPERTRIGLYCERIDEMIA

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ABSTRACT

The current anti-hypertriglyceridemia (HTG) medications still have side effects such as myopathy, rhabdomyolysis, liver damage, and insulin resistance. The development and discovery of new, safer agents to treat hypertriglyceridemia is urgently needed. The leaves of *Murraya paniculata* have been empirically used for lowering lipids. Meanwhile, *Tamarindus indica* leaves are reported to contain high antioxidants and anti-diabetic activity. This study was conducted to evaluate the combination effect of leaf extracts from *Tamarindus indica* and *Murraya paniculata* to treat hypertriglyceridemia in animal models and to predict the optimum ratio. Hypertriglyceridemic Wistar rats were developed by feeding the high fat-sucrose diet for 75 days. The combination of *T. indica* extract (TIE) and *M. paniculata* extract (MPE) was made following the Simplex Lattice Design ranging from 50:350 to 350:50, producing denoted ratios TIE:MPE: (i) 50:350, (ii) 125:275, (iii) 200:200, (iv) 275:125, and (v) 350:50. The extract was given to hyperlipidemic animals orally once per day for 14 days at a dose of 400 mg/kgBW. Prior to the in vivo assay, both extracts were analyzed for their phytochemical content (mineral, phenol and flavonoid content) and water content. The result of lipid profile measurements showed that the combination of 350:50 mg/kgBW (TIE:MPE) gave the strongest effect in lowering triglyceride ($p<0.05$). As the composition of the TIE extract increased, the TG-lowering effect appeared to strengthen and the body weight gain of rats also decreased in a dose dependent manner. The liver protective activity was observed in all combinations based on hepatic catalase and malondialdehyde measurement. Administration of the combined extract with a high proportion of TIE tends to provide better enhancement of muscle lipoprotein lipase (LPL) activity. In conclusion, it was proven that the combination of *Tamarindus indica* and *Murraya paniculata* leaf extracts significantly reduced triglycerides level in animal models. The optimum effect was achieved at a ratio of 350:50 mg/kgBW (TIE:MPE). Finally, purification of extract from primary fat components is suggested for further research.

Key words: anti-hypertriglyceridemia, *Tamarindus indica*, *Murraya paniculata*, leaf extract, polyherbal, Simplex-Lattice-Design, obesity, animal-model

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INTRODUCTION

Along with hypertension, age, genetics, and lifestyle factors such as smoking, food habits, and physical activity level, hyperlipidaemia is a risk factor for cardiovascular diseases. Hyperlipidaemia is characterized by a rise in plasma cholesterol, triglycerides, or both [1]. Acute pancreatitis, insulin sensitivity, and non-alcoholic fatty liver disease (NAFLD) are all consequences of uncontrolled hyperlipidaemia, particularly hypertriglyceridemia (HTG) [2–5].

Until now, anti-HTG medications (statins, niacin, fibrates) are known for their side effects such as myopathy, rhabdomyolysis, liver damage, and insulin resistance [6]. The strategy to reduce TG levels is to inhibit synthesis, prevent absorption, and increase TG utilization in peripheral tissue [7]. However, an increase in TG oxidation in the tissue will have an impact on increasing dangerous reactive oxygen species (ROS) production [8]. Therefore, additional combinations or supplements are needed that can ward off this oxidative stress.

Two tropical plants that have lipid-lowering activity are Tamarind (*Tamarindus indica*) and Orange Jasmine (*Murraya paniculata*). The leaves of Tamarind had been used by Indonesian people as *Jamu Sinom* – a traditional drink – to increase body vitality and accelerate post-illness recovery, as well as for folk medicine in the Caribbean [9–12]. Previous research revealed that Tamarind leaves exhibited antioxidant, cholesterol-lowering, anti-lipase, hepatoprotective, and anti-diabetic activity [13–15]. Meanwhile, Orange Jasmine leaves was reported to contain coumarin compounds, including murmeranzine, isopropylidine murrangatin, murralonginal, and pranferin, as well as polymethoxylated -flavones, -flavonones, and -chalcones [16,17]. Pharmacologically, *M. paniculata* was reported to have hypolipidaemic and hypoglycaemic action, antioxidants, and increased insulin secretion [17,18]. Another study also reported that extracts containing polymethoxylated flavones improved glucose and lipid metabolism [19].

Hypertriglyceridemia conditions will trigger cellular oxidative stress, inactivation of insulin signalling, as well as non-alcoholic fatty liver diseases and acute pancreatitis. To the best of our knowledge, no studies have been published that demonstrate the triglycerides-lowering action of *T. indica* leaves in combination with *M. paniculata* leaves *in vitro* or *in vivo*. This research was designed as a step to find a combination formula of the two plants to treat hypertriglyceridemia. It is hoped that both extracts would reduce triglyceride level. Meanwhile, *T. indica* leaves will ward off oxidative stress, and *M. paniculata* would improve lipolysis. Consequently, the purpose of this study is to get the optimum formula of two extracts in lowering triglycerides in animal models.

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MATERIALS AND METHODS

Material

The leaves of *T. indica* were procured from Sleman district, Yogyakarta, Indonesia, while *M. paniculata* was sourced from the Research Centre for Medicinal Plants and Traditional Medicines in Tawangmangu, Central Java, Indonesia. Plant identification conducted at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada. The assay kits for lipid (Dumolabs®, Neudorf-Austria) containing CHOD-PAP, GPO-PAP, and HDL precipitate; an LPL Activity Assay Kit (Sigma-Aldrich, Singapore); and MDA and Catalase Assay kits (Abbkine, CA, USA) were acquired from local distributor. A total of 33 male Wistar rats, aged 2 months, served as animal models [20] and were obtained from the Pharmacology and Therapy Laboratory, Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada. The *in vivo* test protocol was approved by the Ethics Commission of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia no. KE/FK/0005/EC/2021.

Extract preparation

The leaf powder was extracted individually using 70% technical-grade ethanol through maceration (1:10 w/v) at room temperature for 24 hours, followed by a single re-maceration step. The resulting filtrate was then dried using a rotary evaporator and aerated over a water bath into dryness. For the in vivo study, each extract was emulsified using 0.5% CMC-Na. Separately, 640 mg of each extract was dissolved in 10 mL of CMC-Na resulting in a solution concentration of 64 mg/mL. Furthermore, both solutions were mixed with the following ratio (TIE:MPE) = Group (i) 2.5:17.5 mL, (ii) 6.25:13.75 mL, (iii) 10:10 mL, (iv) 13.75:6.25 mL, (v) 17.5:2.5 mL. Each 5 mL mixture will contain 160 mg of total extract.

Phytochemical analysis

The extract obtained was analysed on several parameters referring to the Indonesian Herbal Pharmacopoeia (IHP) including loss of drying and ash content (gravimetric), mineral content (ICP-OES), total flavonoid (in quercetin equivalent mg/g) and phenolic content (in gallic acid equivalent mg/g) [21–23].

Design of Experiment and animal preparation

In the in vivo study, the Mixture Design model (Simplex Lattice Design) was employed based on the approach by Hong *et al.* [24]. This design involved two variables (the type of extract) and five variations in composition. The groups were denoted by the TIE:MPE combination ratios: (i) 50:350, (ii) 125:275, (iii) 200:200, (iv) 275:125, and (v) 350:50, with a combined dose of 400 mg/kg body weight (BW) as

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detailed in Table 1. Additionally, two control groups were included—one without extract treatment (normal control) and another representing high triglyceride (HTG) control.

After a 1-week acclimatization period, the rats were weighed (151 ± 15 g) and divided into two groups: the normal diet group (referred to as "Normal Control," consisting of 4 animals) and the high-fat diet group (HFD, comprising 29 animals). The HFD formulation included 80% basal feed (CitraFeed®, Indonesia), 15% butter, and 5% duck egg yolk [25]. Rats were maintained on the HFD for 75 days, during which the average triglyceride (TG) value exceeded 270 mg/dL [26]. At the end of the induction period, 4 animals were randomly selected and sacrificed for examination as HTG control.

The remaining 25 rats were randomly assigned to 5 groups. The extract was orally administered to the rats for 14 days at a mixed dose of 400 mg/kg BW, replacing their regular diet with a normal one. After the extract treatment, the animals' blood was extracted, and they were humanely sacrificed using a ketamine/ xylazine cocktail. Serum was obtained by centrifuging non-heparinized blood at 1,000 G for 10 minutes, followed by separation and storage at -21°C prior to testing. Parameters, including lipid profile, hepatic malondialdehyde (MDA), catalase activity, and muscle lipoprotein lipase (LPL), were quantified. Additionally, a histopathological examination of the liver was conducted.

Measurement of lipid profile

Total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides (TG) were determined by enzymatic colorimetric method (CHOD-PAP and GPO-PAP assay) according to commercial kit protocol (Dumolabs®). Non-HDL lipoprotein parameters were obtained by subtracting HDL cholesterol from total cholesterol levels. Plasma lipid levels were expressed as mg/dL.

Measurement of muscle lipoprotein lipase activity

Muscle tissue samples were weighed and homogenized in 0.01 M phosphate-buffered saline (pH 7.4). The resulting homogenate underwent centrifugation at 10,000 G and 4°C for 15 minutes, yielding a supernatant. A Reaction Mix was prepared by combining 194 µL of LPL assay buffer with 1 µL of Substrate Emulsion (MAK109A, Sigma-Aldrich). Subsequently, 5 µL of the supernatant was subjected to reaction with 195 µL of the Reaction Mix following the established protocol. After a 15-minute incubation, fluorescence measurements were taken at excitation (λEx) = 370 nm and emission (λEm) = 450 nm. Standard curves were generated by diluting the Standard LPL across a series of concentrations ranging from 0 to 6 nmol/0.2 mL.

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Measurement of hepatic catalase

A small portion of the liver was taken, weighed and homogenized in phosphate buffered saline (0.01 M, pH 7.4). The homogenate was centrifuged at 10,000 G at 4 °C for 15 minutes and the supernatant was separated. For catalase activity assay, a total of 20 µL of the supernatant was reacted with the reagent kit sequentially according to the protocol. After incubation, the absorbance of each well was read at 540 nm. The absorbance reading was corrected with a blank. One unit of activity was expressed as the amount of enzyme that produces 1.0 nmol formaldehyde per minute at 25 ^{o}C.

Measurement of hepatic malondialdehyde (MDA)

The malondialdehyde assay was conducted using the Micro Lipid Peroxidation Assay kit (Abbkine, Inc.). Malondialdehyde, a marker of lipid peroxidation, was quantified in hepatic homogenate supernatant (100 µL) or PBS (blank) mixed with a Reaction Mix (300 µL). The resulting mixture underwent incubation at 95°C for 30 minutes, followed by cooling to room temperature and centrifugation at 10,000 G for 10 minutes. Afterwards, the supernatant's absorbance was measured at 532 nm and 600 nm. Lipid peroxidation levels were expressed as nmol MDA/mg tissue.

Histopathological examination

After thorough cleaning, the liver was subsequently fixed in a 10% formalin solution. Gradual dehydration with alcohol followed, and the organs were then sectioned into paraffin blocks using a microtome. Finally, organ slides were stained with Haematoxylin and Eosin and examined under a microscope.

Data analysis

The acquired data underwent statistical analysis using Minitab Statistical software. Group differences were assessed via ANOVA at a 95% confidence level, followed by t-tests to determine significance between groups. To ascertain the presence or absence of interaction between the two extracts, a non-linear regression analysis was conducted on the Simplex Lattice Design (SLD) variables, expressed as:

$$Y = a.[A] + b.[B] + c.[A].[B]$$

Here, [A] and [B] represent the concentrations of *T. indica* and *M. paniculata* leaf extracts, respectively, while Y denotes the observed dependent variable.

RESULTS AND DISCUSSION

The objective of this study is to investigate the combined efficacy of *T. indica* (TIE) and *M. paniculata* (MPE) leaf extracts in lowering hypertriglyceridemia status and

defending the liver from HTG circumstances. In this study, the leaves of both plants were extracted and analyzed for extract quality (loss of drying, ash content, total phenolic and flavonoids), and safety (heavy metal content). After that, the two extracts were mixed by varying the composition in the form of a CMC-Na emulsion. The combination of extracts was given to hyperlipidemic mice induced by a high fat diet.

Extracts' properties

The physical characteristics of the extracts observed included Loss of Drying (LoD), ash content (Table 2), and metaloid content (Table 3). The LoD describes the content of volatile substances (water and volatile compounds) in the extract, while the ash content describes the total mineral content. The ash contents of the two extracts were 6.7% (TIE) and 2.2% (MPE). The measurement of total phenolic and flavonoid levels found that the phenolic content in the TIE was 559 mg/g GAE while in the MPE was 258 mg/g GAE. On the contrary, the measurement of total flavonoid levels by spectrophotometry ($AlCl_3$ complex reaction) in the TIE was much lower (12.7 mg/g QE) than MPE (43.2 mg/g QE) (Table 2).

The presence of heavy metals in natural ingredient extracts is a growing concern in traditional medicine development. These metals can be toxic as they disrupt various normal biochemical and metabolic processes. Excessive consumption of dietary heavy metals has been associated with several health issues, such as reduced immunity, heart problems, fetal malformations, and impaired psychosocial and neurological functions [27]. In general, the quality of the extracts tested in this study was sufficient to meet the requirements based on standard extract parameters in the Indonesian Herbal Pharmacopoeia. Phytochemically, it has been confirmed that Tamarind leaves contain high levels of flavonoids and phenolics. Meanwhile, *M. paniculata* leaves contain more flavonoids than Tamarind. This shows that the flavonoid compounds in *M. paniculata* are substituted or methoxylated flavonoids.

The effect of combined extracts on lipid metabolism

Results from the in vivo study indicate that the administration of the combined extract led to a significant reduction in triglyceride (TG) levels ($p < 0.05$), as observed through paired t-tests comparing pre- and post-extract administration (Table 4). Notably, as the composition of the TIE extract increased, the TG-lowering effect appeared to strengthen. Furthermore, lipid parameters reliant on cholesterol measurements revealed significant reductions in non-HDL cholesterol levels ($p < 0.05$) across all groups following combined-extract administration. Interestingly, with the increase in the proportion of TIE in the formula, the body weight gain of rats also decreases in a dose dependent manner. Additionally, the study also demonstrated

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ISSN 1684 5374

that the decrease in TG levels corresponded to a normalization of the Atherogenic Index (AI). The AI, calculated from the logarithmic molar ratio of TG to HDL, is categorized as low cardiovascular risk (-0.3 – 0.1), medium risk (0.1 – 0.24), or high risk (> 0.24) [28].

In the present study, the combination extract consistently reduced serum triglyceride (TG) levels across all experimental groups. As the proportion of TIE increased, the TG-lowering effect intensified in a dose-dependent manner (Table 4). These findings suggest that the phytochemical components within the TIE extract primarily contribute to the observed reduction in TG levels. This aligns with previous research demonstrating the lipid-lowering properties of flavonoids found in tamarind leaves. It was proposed that the TIE extract may enhance hormone-sensitive lipase (HSL) expression while down regulating mRNA expression of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), diacylglycerol acyltransferase (DGAT), and HMG-CoA reductase [29].

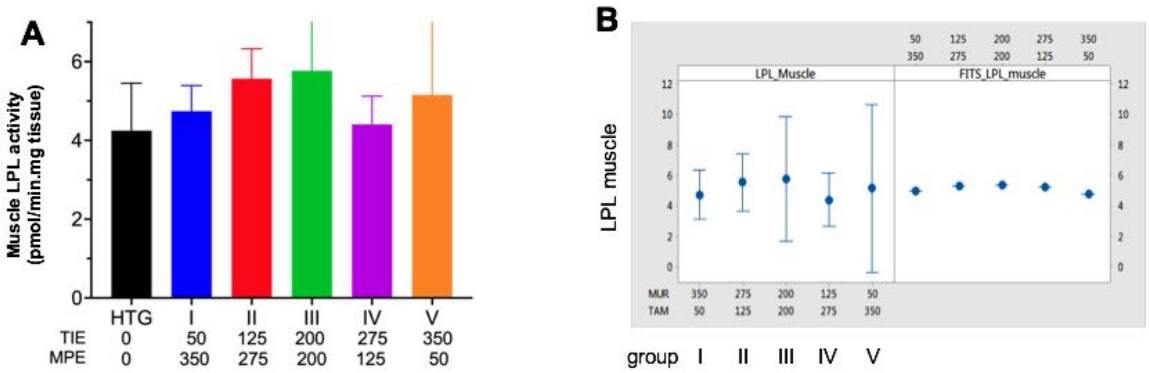

Figure 1: The effect of combined leaf extracts of *Tamarindus indica* and *Murraya paniculata* on muscle lipoprotein lipase (LPL) activity. (A) LPL activity of peripheral muscle tissue. Muscle LPL appeared to increase in all groups as compared to the HTG group. (B) Non-linear regression of muscle LPL activity against the composition of the extract combination. (p>0.05, n=3)

To enhance triglyceride clearance, one strategy involves increasing the expression or activity of muscle lipoprotein lipase (LPL). In this study, the impact of combined extracts on muscle LPL activity was investigated. As depicted in Figure 1, the measurement results revealed a slight increase in muscle LPL activity across all combined-extract groups ($p > 0.05$) compared to the high-triglyceride (HTG) group (4.2 pmol/min.mg tissue). Among the extract groups, muscle LPL activity ranged from 4.4 to 5.8 pmol/min.mg tissue, with the highest activity observed in group III (TIE 275: MPE 125) at 5.8 pmol/min.mg tissue.

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These results tentatively suggest that administration of the combined TIE and MPE extracts may shift TG metabolism from systemic circulation toward muscle tissue. Previous work by Savage *et al.* highlighted three potential mechanisms for reducing serum TG levels: inhibition of food intake, suppression of hepatic TG synthesis, and enhancement of fat oxidation [4]. Interestingly, the increase in muscle LPL activity was consistent across all combination groups, indicating comparable modulatory effects. Remarkably, apigenin—a phytochemical present in both TIE and MPE extracts—has been implicated in LPL regulation [22,30]. In a prior study, Jung *et al.* reported that apigenin administration stimulated LPL gene expression in hyperlipidemic mice [31]. Wiyono *et al.* further highlighted that *T. indica* leaves contain abundant polyphenols, including vitexin and quercetin [22]. Reportedly, quercetin is able to reduce plasma TG levels by increasing TG uptake into brown adipose tissue [32]. Consequently, the dominant TG-lowering effect of TIE over MPE can be attributed to multifaceted mechanisms, impacting TG absorption, synthesis, and deposition.

The effect of combined extracts on liver antioxidant system

In the present study, the administration of a combined extract of TIE and MPE extracts appeared to enhance hepatic catalase (CAT) enzyme activity while reducing hepatic malondialdehyde (MDA) levels (Figure 2). However, these changes did not reach statistical significance ($p > 0.05$). The CAT activity in all extract groups exceeded that of the high-triglyceride (HTG) group (ranging from 6.4 to 7.5 nmol/min.mg tissue), whereas MDA levels in the combined-extract groups were lower than those in the HTG group ($p > 0.05$). Among the extract combinations, the most pronounced decrease in MDA occurred in group V (TIE 350: MPE 50), while group III (TIE 200: MPE 200) exhibited the weakest effect.

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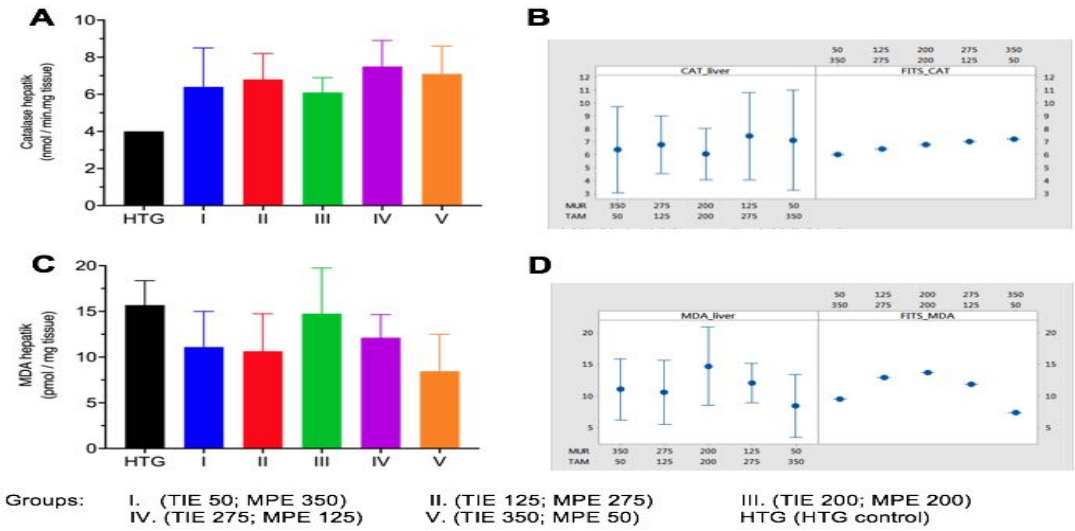

Figure 2: The effect of combined extracts of *Tamarindus indica* and *Murraya paniculata* leaves on hepatic tissue oxidative stress. Administration of the extract to hyperlipidemic rats increased catalase enzyme (CAT) activity and decreased malondialdehyde (MDA) levels in all test groups ($p > 0.05$, $n = 3$)

(A) Comparison of hepatic catalase activity values between test groups.
(B) Non-linear regression curve of catalase activity on the composition of the extract combination.
(C) Comparison of MDA levels in liver tissue, expressed in pmol/mg tissue.
(D) Regression curve of MDA levels on the composition of the extract combination.
(Remarks: TIE (*T. indica* leaf extract); MPE (*M. paniculata* leaf extract))

Catalase is a defense enzyme that converts hydrogen peroxide into water and oxygen, while MDA serves as a marker of lipid peroxidation. Flavonoids play a crucial role in inhibiting lipid peroxidation by donating hydrogen, thereby neutralizing radical molecules [33]. Interestingly, administration of combined extracts with a high proportion of TIE appeared more effective in reducing MDA levels and enhancing catalase activity.

Previous research demonstrated that quercetin significantly restored pancreatic and liver damage by modulating the antioxidant defense system [34]. Specifically, it increased superoxide dismutase (SOD) and reduced glutathione (GSH), while also inhibiting inflammatory progression through regulation of NF-kB, TNFα, and IL-6 gene expression. Another study conducted by Sulimani *et.al.* [35] also stated that quercetin was able to protect the liver from cellular damage caused by celecoxib.

Based on histopathological observations (Table 5), it is evident that animals induced by a high-fat diet and fructose exhibited diffuse hydropic degeneration (HD) in their livers. However, this hydropic degeneration resolved after administration of the

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combined extract. Only one rat in group 4 experienced mild steatosis following combined-extract treatment. In contrast, group V (TIE 350: MPE 50) showed hepatic glycogenosis (HG) in some animals (Figure 3). Hepatic glycogenosis is a condition commonly observed in individuals with diabetes mellitus or non-alcoholic fatty liver disease (NAFLD) [36]. The interplay of elevated insulin activity and high sugar levels is believed to stimulate excessive glycogen synthesis [37].

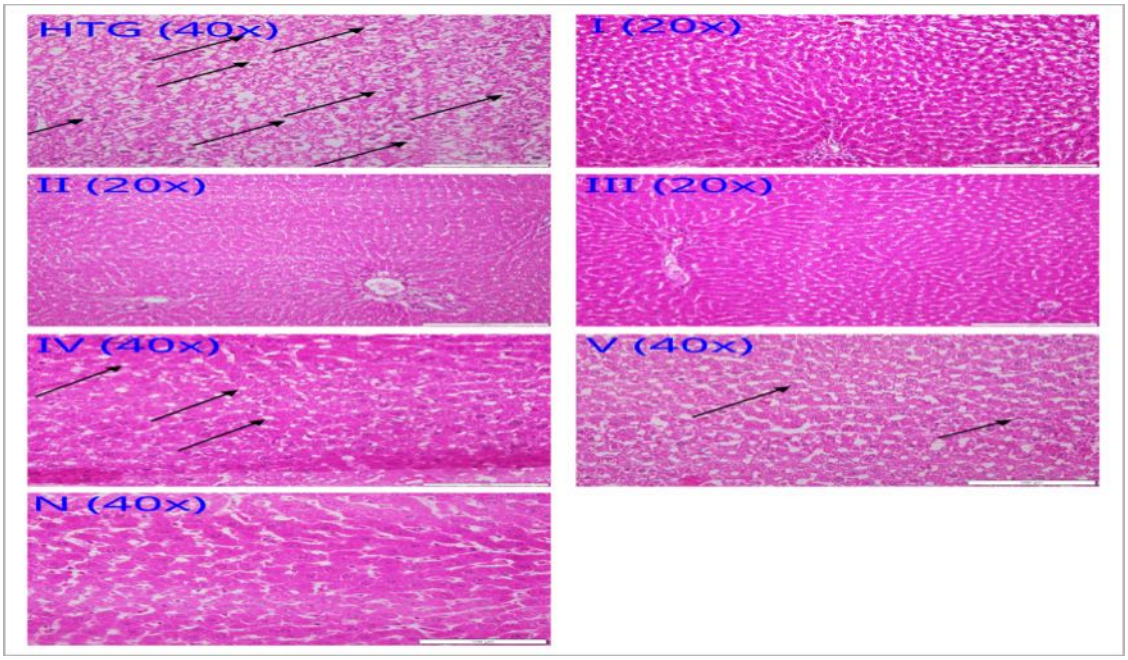

Figure 3: Histopathological picture of the liver of a hyperlipidemia animal model after the administration of a combination extract of *Tamarindus indica* and *Murraya paniculata* leaves. (HTG) Liver tissue with hydropic degeneration (HTG control group). Arrows indicate accumulated water. (I-III, N) Liver tissue without pathological alteration (group I, II, III, and Normal control). (IV) Mild steatosis found in group IV. Arrows indicate lipid droplets. (V) Liver tissue with glycogenosis. Arrows indicated accumulated glycogen

Predicted optimum combination for TG lowering

In this study, an HTG-case modelling using statistical methods was conducted to estimate the optimal predictive composition for HTG cases. The therapeutic goal for HTG management involves reducing triglyceride (TG) levels, non-HDL cholesterol, and enhancing antioxidant enzyme activity (specifically catalase) and muscle lipoprotein lipase (LPL).

The Response Optimization within the Simplex Lattice Design (SLD) analysis was employed to evaluate parameters such as TG levels, non-HDL, muscle LPL activity,

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catalase, and malondialdehyde (MDA) (Figure 4). This approach draws inspiration from Ghosh *et al.*, who utilized Response Surface Methodology (RSM) and Response Optimization computational modeling to determine the optimal dose of streptozotocin for inducing neuroinflammation [38]. Specifically, the Simplex Lattice Design (SLD) and Response Optimization (RO) were utilized to identify the optimal mixture composition for anti-hypertriglyceridemia extracts. The optimization allows researchers to pinpoint combinations of independent variables that collectively influence one or more responses [39,40]. In essence, RO combines several regression curves to determine the desired target value for each dependent variable, whether aiming for the highest, lowest, or a specific value.

Based on the analysis results, the optimum predictive combined extract composition for HTG cases was 350:50 (TIE:MPE) within the tested range of 50-350 mg/kg BW. This finding underscores the potency of the TIE extract in reducing triglycerides.

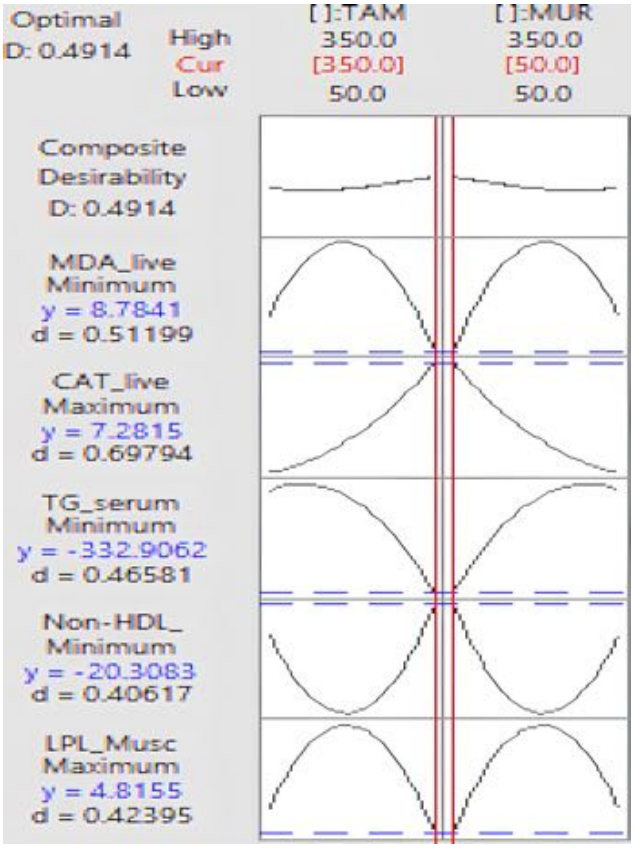

Figure 4: Response Optimization analysis of the composition of the combined extract of *Tamarindus indica* and *Murraya paniculata* leaves on hypertriglyceridemia. Optimum composition was achieved at 350:50 mg/kg BW (TIE:MPE)

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CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The combined extract of *T. indica* and *M. paniculata* leaves effectively reduced hypertriglyceridemia in animal models, particularly by modulating muscle lipoprotein lipase resulting in the reduced triglycerides and non-HDL cholesterol levels. It also provided protection against oxidative stress and cellular injury in the liver. The optimal composition was achieved at 350:50 mg/kg BW (*T. indica*: *M. paniculata*). Consequently, this finding proves that the combination of *T. indica* and *M. paniculata* leaf extracts has the potential to be developed as a polyherbal to reduce the risk of heart disease in humans. It is recommended to carry out purification in further research, especially to remove primary fat components from the extract.

ACKNOWLEDGEMENTS

The researcher thanked Taufik Kurniawan for his help in preparing high fat feed. This research was funded by the Final Project Recognition Grant Universitas Gadjah Mada Number 5075/UN1. P. II/Dit-Lit/PT.01.01/2023.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Table 1: Design of Experiment for *in vivo* study (*Simplex Lattice Design*)

Group	Ratio		Doses (mg/kgBW)			Subject rat
	X1	X2	*T. indica* (X1)	*M. paniculata* (X2)	Total	
I	0.125	0.875	50	350	400	HTG
II	0.312	0.688	125	275	400	HTG
III	0.500	0.500	200	200	400	HTG
IV	0.688	0.312	275	125	400	HTG
V	0.875	0.125	350	50	400	HTG
Normal	0	0	0	0	0	Normal
HTG	0	0	0	0	0	HTG

Table 2: Characteristics of *Tamarindus indica* and *Murraya paniculata* leaves extracts

Parameter	*T. indica*	*M. paniculata*
Loss of Drying (%)	25.1 ± 0.5	29.1 ± 0.05
Ash content (%)	6.72 ± 0.17	2.18 ± 0.08
Phenols (GAE mg/g)	559.0 ± 16.0	258.5 ± 12.9
Flavonoids (QE mg/g)	12.7 ± 1.1	43.2 ± 0.4

Table 3: Minerals content in *Tamarindus indica* and *Murraya paniculata* leaves extracts

Metal content (µg/g) **)	Metals								
	Cd	Hg	Pb	Co	Cr	Mn	Fe	Al	Zn
T. indica	*	*	*	*	*	13.2 ± 0.1	9.6 ± 0.4	3.2 ± 1.4	*
M. paniculata	*	*	*	*	*	18.6 ± 0.1	14.3 ± 0.1	*	7.3 ± 0.3

* Below Limit of Detection

** Limit of Detection, LOD = 0.021 ppm

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SCHOLARLY, PEER REVIEWED
Volume 24 No. 9
September 2024
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 4: Lipid profile and body weight gain (14 days) of hyperlipidemic animal models between before and after administration of a combination of *Tamarindus indica* and *Murraya paniculata* leaves extracts. (Mean ± SD, n ≥ 4)

Parameter	Group						
	I	II	III	IV	V	Normal	HTG
TG (mg/dL)							
Pre	274 ± 73.2	211 ± 165	279 ± 112	347 ± 56	417 ± 258		
Post	76.5 ± 21.5	57 ± 24.1	52.4 ± 8	107 ± 51	82 ± 25.7	110 ± 32	274 ± 111
p-value	0.002	0.041	0.002	0.001	0.017		
TC (mg/dL)							
Pre	78.7 ± 10.5	80.6 ± 13.8	79.3 ± 11.6	86.7 ± 7.3	77.7 ± 8.5		
Post	62.9 ± 11.8	63.8 ± 17.2	54.9 ± 12.5	64.9 ± 12.2	62 ± 13.6	105.4 ± 8.6	72.7 ± 13.9
p-value	0.03	0.02	0.002	0.02	0.03		
HDL (mg/dL)							
Pre	30.8 ± 2.6	32.4 ± 5.9	30.2 ± 6.9	34.1 ± 2.7	31.5 ± 5.8		
Post	38.4 ± 5.7	39.2 ± 8.2	33.2 ± 5.7	39.3 ± 6.7	34.9 ± 7.6	44.7 ± 2.3	25.5 ± 1.4
p-value	0.019	0.057	0.118	0.075	0.288		
Non-HDL (mg/dL)							
Pre	47.9 ± 8.9	48.2 ± 10.2	49.1 ± 5.5	52.6 ± 5.5	46.2 ± 7.5		
Post	24.5 ± 6.3	24.6 ± 10.4	21.7 ± 8.2	25.6 ± 6	27 ± 7	60.7 ± 6.8	47.2 ± 14.7
p-value	0.002	0.002	0.001	0.001	0.001		
Atherogenic Index*							
Pre	0.5 ± 0.2	0.3 ± 0.3	0.5 ± 0.3	0.6 ± 0.1	0.6 ± 0.4		
Post	-0.1 ± 0.1	-0.3 ± 0.2	-0.2 ± 0.1	-0.0 ± 0.2	-0.1 ± 0.1	-0.1 ± 0.1	0.7 ± 0.2
Body Weight (g)							
Pre	159.2 ± 33.5	135 ± 39.2	153 ± 40.8	188.2 ± 32	179.4 ± 56.3	285 ± 16	
Post	180.8 ± 32.2	152.8 ± 40.2	166.3 ± 29.8	193.8 ± 43.3	186 ± 63.8	274 ± 14.9	
BW gain	21.6 ± 12.4	17.8 ± 4.4	13.3 ± 14.8	5.7 ± 16.7	6.6 ± 16.5	-11 ± 5.5	

Note: * Atherogenic Index = log (mol TG/ mol HDL)
Treatment groups (*T. indica* + *M. paniculata*) = I (50 + 350), II (125 + 275), III (200 + 200), IV (275 + 125), V (350 + 50), in mg/kg BW

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ISSN 1684 5374

Table 5: Histopathology of liver and pancreas from hyperlipidemic rat after administration of combined leaves extracts of *Tamarindus indica* and *Murraya paniculata*. (NPA: no-pathological alteration)

Group	No	Liver
HTG	1	Diffuse hydropic degeneration
(n = 4)	2	Diffuse hydropic degeneration
	3	NPA
	4	Diffuse hydropic degeneration
N	1	NPA
(n = 4)	2	NPA
	3	NPA
	4	NPA
I	1	NPA
(n = 5)	2	NPA
	3	Glycogen accumulation
	4	NPA
	5	NPA
II	1	NPA
(n = 5)	2	NPA
	3	NPA
	4	NPA
	5	NPA
III	1	NPA
(n = 5)	2	NPA
	3	NPA
	4	NPA
	5	NPA
IV	1	Mild steatosis
(n = 5)	2	NPA
	3	NPA
	4	NPA
	5	NPA
V	1	NPA
(n = 5)	2	Glycogen accumulation
	3	NPA
	4	Glycogen accumulation
	5	NPA

Note: Combination groups (*T. indica* + *M. paniculata*) = I (50 + 350), II (125 + 275), III (200 + 200), IV (275 + 125), V (350 + 50), in mg/kg BW

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