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Original Research

Effects of the Polyherbal Wattana Formula on Food Intake, Intestinal Transit, and Ileum Contraction in Wistar Rats

Pinpat Tripatara[1], Pruksa Chotitham[1], Tassanee Paraput[2], Pravit Akarasereenont[1,2]*

[1]Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[2]Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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Abstract

The polyherbal Wattana formula (WNF) has been used in traditional practice for over 30 years to promote health and prevent age-related issues. However, no scientific evidence supports its efficacy, including its impact on the gastrointestinal system in older individuals. This study examined the effects of WNF on intestinal transit, body weight, and food intake in Wistar rats that received oral WNF at doses of 100, 300, or 1000 mg/kg for 14 days. Following treatment, the small intestine was isolated, and intestinal transit was assessed via charcoal meal travel distance. Compared to the vehicle, 1000 mg/kg WNF significantly reduced intestinal transit in the charcoal meal test and decreased body weight. Additionally, 600 µg/mL WNF extract impeded acetylcholine-induced ileum smooth muscle contraction *ex vivo*. WNF at all doses demonstrated no effects on food intake. Our findings suggest that WNF may have potential antidiarrheal effects and benefits in weight management and abdominal spasm reduction.

Keywords: Weight loss; Antispasmodics; Food intake; Herbal formula; Intestinal transit

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*Corresponding Author: Pravit Akarasereenont
Department of Pharmacology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
Email: pravit.auk@mahidol.edu

Introduction

The polyherbal Wattana formula (WNF) has been employed in Thai traditional medicine since 1982, for promoting health increasing appetite, and muscle-ache relief [1]. The medicine is in powder and pill dosage forms comprising of 18 components: the fruit of *Aegle marmelos* (L.) Corrêa (bael fruit), root of *Aucklandia costus* Falc. (costus), rhizome of *Boesenbergia rotunda* (L.) Mansf. (kra chai), wood of *Caesalpinia sappan* L. (sappan tree), flower of *Carthamus tinctorius* L. (safflower), wood of *Cinnamomum ilicioides* A.Chev. (kha thon), fruit peel of *Citrus sinensis* (L.) Osbeck (sweet orange), root of *Cladogynos orientalis* Zipp. (chetta phang khi), stem of *Cryptolepis dubia* (Burm.f.) M.R.Almeida (thao en on), corm of *Cyperus rotundus* L. (nutgrass), stem of *Derris scandens* (Roxb.) Benth. (thao wan priang), oleo gum resin of *Ferula assa-foetida* L. (asafoetida), rhizome of *Ligusticum sinense* Oliv. (kot hua bua), stem of *Mallotus repandus* (Rottler) Müll.Arg. (kho khlan), seed of *Piper nigrum* L. (pepper), leaf of *Putranjiva roxburghii* Wall. (ma khum kai), fruit of *Terminalia chebula* Retz. (myrobalans) and stem of *Tinospora crispa* (L.) Hook.f. & Thomson (bora phet) [1] Under traditional herbal principles, the main WNF components are considered hot taste herbs [1], including *Cinnamomum ilicioides* A. Chev., *Cladogynos orientalis* Zipp., *Cyperus rotundus* L., *Ferula assa-foetida* L., and *Piper nigrum* L. Moreover, the components of WNF consist of bitter taste and sour taste including *Tinospora crispa* (L.) Hook.f. & Thomson., *Putranjiva roxburghii* Wall., and *Terminalia chebula* Retz..

From the analysis of the recipe's herbal components, there are hot-tasting herbs that help to improve digestion, make the digestive system good and balanced, and good bowel function, due to the traditional medicine concept of easily moving the air element by hot tasting herbs. Its individual properties do not match the traditional use. Moreover, prior studies have reported WNF component effects of increased metabolism, reduced ileum contraction, and inhibited intestinal transit (Table 1). Earlier research demonstrated that gallic acid presented in WNF [2] promoted weight loss in rats [3]. Therefore, this study aimed to examine the impact of WNF on gastrointestinal system through the measures of intestinal transit, ileum contraction, food intake, and body weight in Wistar rats, to verify the traditional indication and perhaps investigate the new efficacy of WNF.

Materials and Methods

Animals

60 male Wistar rats (200–250 g), aged 7–8 weeks, were purchased from the National Laboratory Animal Center, Nakhon Pathom, Thailand. The animals were given a standard diet and water ad libitum and housed in the Faculty of Medicine Siriraj Hospital's animal care facility under standard conditions (22–24 °C and 12-h light/dark cycle). The rats were acclimated for a minimum of 1 week before experimentation. At the end of the experiment, the rats were humanely euthanized by exsanguination [4]. The study protocol followed the animal welfare code of conduct, 3R concepts, and National Research Council Guide for the Care and Use of Laboratory Animals [5] and received approval under certificate number 007/2559 from the Siriraj Animal Care and Use Committee, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The work complies with the ARRIVE guidelines [6].

Drugs and chemicals

Olanzapine was procured from Eli Lilly (Spain), while activated charcoal, gum acacia, starch, acetylcholine chloride, NaCl, KCl, $MgCl_2$, $CaCl_2$, NaH_2PO_4, $NaHCO_3$, glucose, and atropine were obtained from Sigma–Aldrich (USA). All chemicals and drugs were of analytical grade. They were dissolved in distilled water to create fresh solutions on experimental days.

For the metabolomics profiling study, methanol (LC-MS optima grade) was purchased from Fisher Chemical (USA). Caffeine (3-METHYL-13C, 99%), cholic acid (2, 2, 4, 4-D4, 98%), and leucine enkephalin (C28H-37N5O7) were obtained from Sigma–Aldrich (USA). Formic acid (LC-MS grade) was sourced from Scharlau (Spain), and purified water was prepared using a Milli-Q water system (Millipore, France).

Plant materials and WNF extract preparation

All herbal components were sourced from qualified suppliers in Thailand. Production procedures were supported by the Herbal Medicines and Products Manufacturing Unit, Center of Applied Thai traditional Medicine, Faculty of Medicine Siriraj Hospital, Bangkok. The procedures adhered to current good manufacturing practice certification. In brief, individual herbs were authenticated by experts, including certified pharmacognosists from the Center of Applied Thai traditional Medicine and the production processes were certified from GMP / PICs (GMP Pharmaceutical Inspection Co-operation Scheme), to ensure the quality of herbal identification and production. Raw materials were washed with de-ionized water, dried in a hot-air oven, ground, sieved, weighed equally, and packed as mixed herbs in laminated vacuum packaging bags. Then, the WNF extraction was prepared by 80% ethanol at ratio of 1:10 (w/v) using ultrasonic sonicator for 3 times (30 min each time). The extraction solution was filtrated using filter paper and the residue was evaporated by rotary evaporator before lyophilized [7]. The yield of extraction was 13.8% (w/w). Ethanol (50%) was

Table 1. Summary of the effects of each component in WNF

No.	Scientific Name	Effects of components
1	*Aegle marmelos* (L.) Corrêa	Metabolism increase [39]
2	*Aucklandia costus* Falc. synonymous: *Aucklandia* lappa Decne.	Antidiarrheal [55], decrease intestinal transit, carminative [56], anti-inflammatory bowel disease [50], loss of appetite, indigestion, diarrhea, abdominal pain [57], antispasmodic activity [51, 57], inhibitory activity the gastrointestinal dynamic mechanism [51]
3	*Boesenbergia rotunda* (L.) Mansf. synonymous: *Boesenbergia pandurata* Schltr.	Decrease food intake [58], no effect on body weight [59], increase metabolism [40], carminative [45, 60]
4	*Caesalpinia sappan* L.	Decrease intestinal transit, antidiarrheal [62]
5	*Carthamus tinctorius* L.	Metabolism increase [41]
6	*Cinnamomum ilicioides* A.Chev.	-
7	*Citrus sinensis* (L.) Osbeck	-
8	*Cladogynos orientalis* Zipp.	Carminative [62]
9	*Cryptolepis dubia* (Burm.f.) M.R.Almeida	-
10	*Cyperus rotundus* L.	Decrease food intake and body weight [63], decrease ileum contraction [16], decrease intestinal transit [64], antidiarrheal [65]
11	*Derris scandens* (Roxb.) Benth.	No effect on body weight [66], antidiarrheal [67]
12	*Ferula assa-foetida* L.	Decrease body weight [68], decrease ileum contraction [17], carminative [48, 69], antispasmodic activity [48]
13	*Ligusticum sinense* Oliv.	Antispasmodic activity, analgesic activity [52]
14	*Mallotus repandus* (Rottler) Müll.Arg.	-
15	*Piper nigrum* L.	Decrease body weight, increase metabolism [25], decrease ileum contraction [18], decrease intestinal transit [24], carminative [70], anti-diarrhea [72], antispasmodic activity [71], reduce high fat diet [49]
16	*Putranjiva roxburghii* Wall.	-
17	*Terminalia chebula* Retz.	Decrease body weight [72], laxative [46, 73], antispasmodic activity [47]
18	*Tinospora crispa* (L.) Hook.f. & Thomson	No effect on food intake [74], decrease body weight [42, 75], increase metabolism [42], antispasmodic activity [54]

used as solvent to prepare WNF extract solution used in the experiment.

The impact of WNF on intestinal transit distance in Wistar rats

The rats were divided into 5 groups (5 groups of rats, n = 6 per group) (Figure 1). The 3 treatment groups received daily doses of 100, 300, or 1000 mg/kg WNF in 3 mL sterile water intragastrically for 14 days. The used doses and dosage forms are translated from the human dose stating in Investigator's Brochure [1], 1000 mg 3 times daily before meal (3000 mg/day), to rat dose of 300 mg/kg/day through body surface area formula. The negative control group received only sterile water intragastrically for 14 days, while the positive control group received sterile water for 14 days and 0.05 mg/kg atropine on day 15 [8]. The rats fasted for 18 hours with free access to water before the final treatment on day 15 [9]. Upon 2 hours post-treatment, each animal was orally gavaged with 1 mL freshly prepared charcoal meal (activated charcoal: gum acacia: starch = 10:10:20 mg/100 mL water) [10], and a 30-min timer was set. After 30 min, rats were anesthetized using 50 mg/kg ketamine and 5 mg/kg xylazine, administered intramuscularly, and humanely euthanized. Organ segments from the stomach to the cecum were excised. Charcoal meal distances relative to the total small intestine length were cal-

culated using the formula %transit = (charcoal meal traveled distance/total small intestine length) x 10 [11].

The impact of WNF on acetylcholine (ACh)-induced contraction of isolated Wistar rat ileum

The rats were anesthetized using 50 mg/kg ketamine and 5 mg/kg xylazine mixture, administered intramuscularly [12], and humanely euthanized. A 2 cm-long terminal ileum section was excised and suspended in an organ bath containing 20 mL of Tyrode's solution, which was continuously oxygenated. Ileum contractions were recorded with a Harvard isotonic transducer at a resting tension of 1 g. Following a 30-min equilibration period with a stable baseline, treatments (6 groups of rats, n = 4 per group (Figure 1); ACh, 50% ethanol vehicle, atropine, or 60, 200, or 600 µg/mL WNF extract) were added to the organ bath and incubated for 3 min. The amount of 1 mL of 10 µM ACh was used to induce ileum contractions [13]. They were measured as maximal tension changes from the predrug baseline using the PowerLab system and LabChart program (ADInstruments, Australia). The doses of WNF had been chosen from the 13.8% yield of the middle dose of crude drug in the intestinal transit part (300 mg/kg) with the concentration reported in standard model.

The impact of WNF on food intake and body weight in Wistar rats

Food intake and body weight were recorded daily in the rats used for the intestinal transit experiment. A positive control group (n = 6) received 3 mg/kg olanzapine daily for 14 days [14]. Each cage received 100 g of food, and after 24 h, leftover food was weighed; the deficit was calculated as food intake. Rats were sacrificed as described in the intestinal transit experiment.

Metabolomics profiling

Liquid chromatography (LC) conditions utilized in a Waters ACQUITY UPLC System (Waters Corp., USA) with an ACQUITY HSS T3 column (1.8 µm; 2.1 x 100 mm) at 40 °C. The mobile phase comprised 0.1% formic acid in purified water (A) and 0.1% formic acid in MeOH (B). The gradient program was as follows: 0 min, 100% A; 16 min, 100% B; 20 min, 100% A; 24 min, 100% A. The total run time per analysis was 24 min, with an injection volume of 5 µL and a flow rate of 400 µL/min [15].

Metabolomics profiling analysis of plasma samples was performed using a Waters XevoTM QTOF mass spectrometer with an electrospray ionization (ESI) source. Analysis occurred in positive-ion (ESI+) and negative-ion (ESI−) modes, employing elevated energy MS (MSE) mode with low and high collision energies of 4 V and 20 V, respectively. The mass range was set at 100–1200 Da in-continuum data type. The source parameters were 3 kV electrospray capillary voltage, 40 V cone voltage, 150 °C source temperature, and 500 °C desolvation temperature. The cone and desolvation gas flows were 50 L/h and 1000 L/h, respectively. Mass data were corrected during acquisition using an external reference (LockSpray) of leucine enkephalin solution at a 10 µL/min flow rate and 20-second scan injection, generating a reference ion for ESI+ mode ([M + H]+, m/z 556.2771) and ESI− mode ([M - H]−, m/z 554.2615). Pooled QC samples were injected at the beginning of the run, and after every 10 samples to ensure accuracy and reproducibility. Data were acquired using MassLynxTM (V4.1) software [15].

Measurements and statistical analyses

Data are presented as the mean ± standard error of the mean (SEM). Statistical significance was evaluated using one-way and two-way ANOVA, with $P \leq 0.05$ considered significant. GraphPad Prism (version 5) was employed for statistical analysis and graph plotting.

Mass spectral analysis was performed using UNIFI

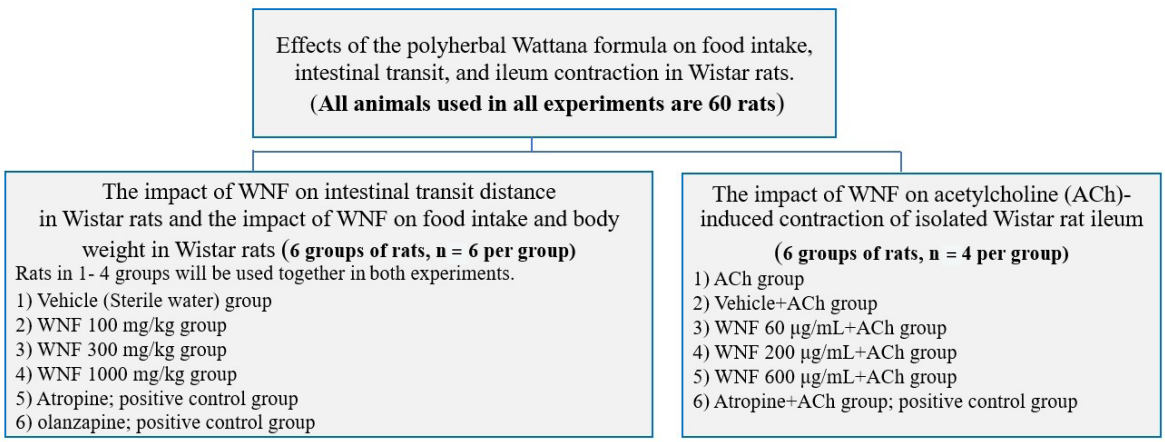

Figure 1. The number of animals to use in the experiment

Scientific Information System software. Multivariate analysis was used for data analysis. The data were stratified into 5 groups: sham; 100 mg/kg, 300 mg/kg, and 1000 mg/kg WNF; and 3 mg/kg olanzapine. Peak intensities were median-normalized and Pareto-scaled. Principal component analysis (PCA) was applied for predictive model development. Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) classified compounds in 2 groups, and the S-plot explained metabolic profile changes in the sham and treatment groups. Metabolites with variable importance in projection values > 1 were selected as the most discriminating features among treatments.

Results

The impact of WNF on ACh-induced contraction of isolated Wistar rat ileum

In the ACh-induced groups, no significant difference was observed between contractions with WNF vehicle (92.17 ± 1.16%) and ACh alone. Incubation with 600 µg/mL WNF significantly reduced ACh-induced contractions by 41.54 ± 3.86% compared to ACh alone. However, incubation with 60 µg/mL and 200 µg/mL WNF did not significantly alter the effect of ACh on ileum contractions (76.50 ± 3.64% and 75.38 ± 3.21%, respectively). Atropine, the positive control, entirely inhibited ACh-induced ileum contractions (Figure 2).

The impact of WNF on intestinal transit in Wistar rats

The charcoal meal propulsion in the rats receiving 1000 mg/kg WNF significantly decreased (55.81 ± 1.33%) compared to the vehicle group (69.78 ± 1.20%). In groups given 100 mg/kg and 300 mg/kg WNF, transit percentages were not significantly different from the vehicle group (61.07 ± 0.80% and 61.04 ± 1.11%, respectively). Atropine, the positive control, significantly inhibited the intestinal transit percentage (13.56 ± 1.30%) of charcoal compared to the vehicle group (Figure 3).

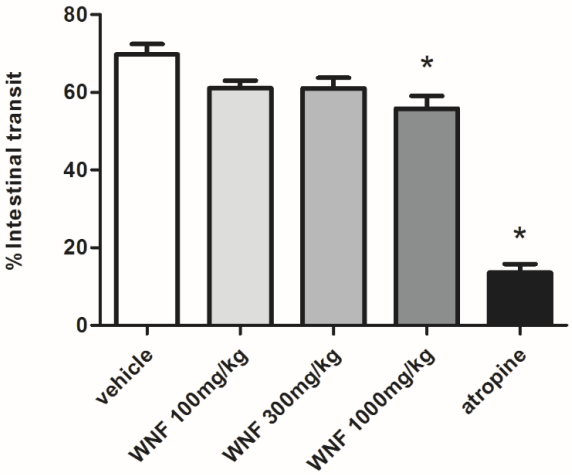

Figure 2. The impact of WNF on ACh-induced contraction of isolated Wistar rat ileum. Treatments (50% ethanol vehicle, or 60, 200, or 600 µg/mL WNF extract) were added directly to the organ bath and incubated for 3 min. The amount of 1 mL of 10^{-5} µM ACh induced ileum contractions. Data are expressed as the means +SEMs. One-way ANOVA shows significance *P ≤ 0.05 compared to ACh only (n = 9).

The effect of WNF on food intake and body weight in Wistar rats

WNF treatments did not significantly affect food intake; whereas olanzapine significantly increased food intake compared to the vehicle group from day 7 to day 14 of treatment (Figure 4).

At the experiment's outset, no significant differences in animal body weights were observed among all groups. However, from day 12 to day 14, the 1000 mg/kg WNF group exhibited a significant decrease in cumulative body weight compared to the vehicle group (Figure 5).

Metabolomics profiling

LC-QTOF analysis was conducted, with chromatogram results displayed as positive and negative ESI modes (Figure 8 and Figure 9). PCA revealed slight differences between the sham and treatment groups (Figure 6A).

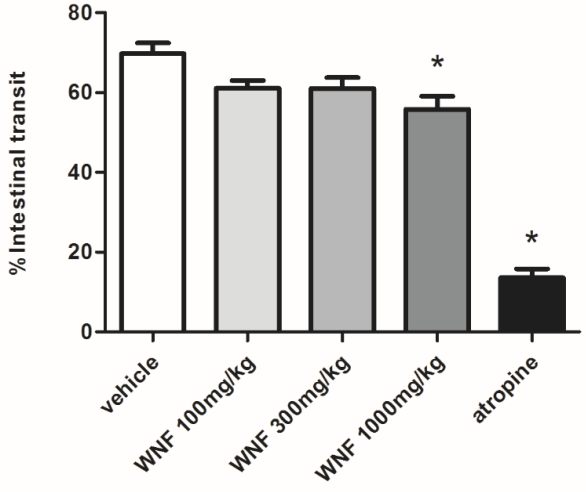

Figure 3. The impact of WNF on intestinal transit in Wistar rats. Wistar rats in the treatment groups received daily doses of 100, 300, or 1000 mg/kg WNF intragastrically for 14 days. The control groups were sterile water as the negative control and atropine on day 15 as the positive control. The rats were then anesthetized and humanely sacrificed, and organ segments from the stomach to the cecum were excised for transit percentage measurements. Data are expressed as the means ± SEMs. One-way ANOVA showed significance; *P ≤ 0.05 compared to the vehicle (n = 6).

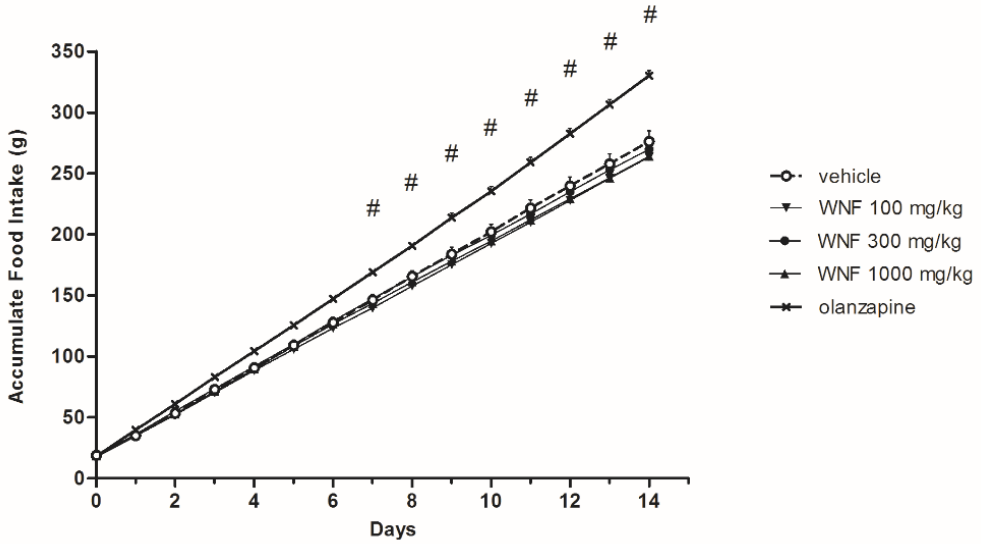

Figure 4. The impact of WNF on food intake in Wistar rats. Accumulated food intake in male Wistar rats after consuming vehicle (sterile water; n = 5), WNF (100, 300, 1000 mg/kg; n = 6), or olanzapine (3 mg/kg; n = 6) for 14 days. Data are expressed as the means ± SEMs. Two-way ANOVA shows significance; *P ≤ 0.05 for vehicle compared to olanzapine.

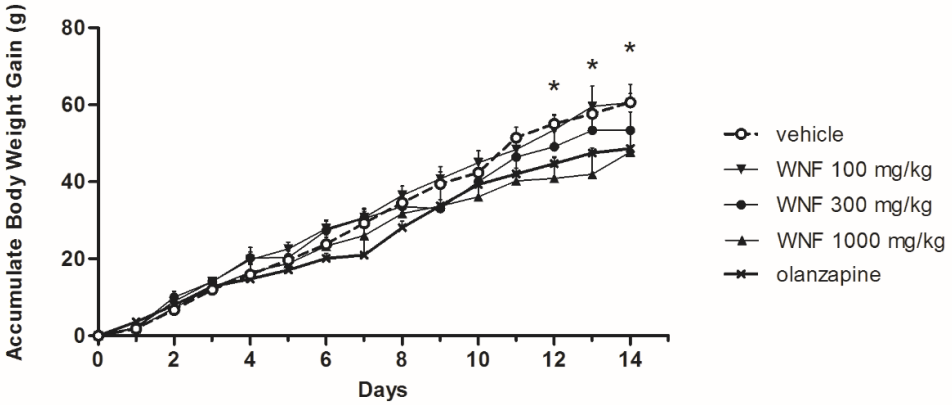

Figure 5. The impact of WNF on body weight in Wistar rats. Cumulative body weight gain in male Wistar rats after consuming vehicle (sterile water; n = 5), WNF (100, 300, or 1000 mg/kg; n = 6), or olanzapine (3 mg/kg; n = 6) for 14 days. Data are expressed as the means ± SEMs. Two-way ANOVA shows significance; *P ≤ 0.05 for vehicle compared to WNF 1000 mg/kg.

Subsequently, the supervised method OPLS-DA was employed to analyze group separation differences. The S-plot demonstrated the reliability of group separation between the vehicle and WNF groups (100, 300, or 1000 mg/kg). Cutoff values of P ≤ 0.05 for magnitude and P > 0.1 for reliability in the S-plot were used to select biomarkers explaining metabolites of interest under different experimental conditions (Figure 6B).

Eighty-three metabolites were identified in Wistar rats (56 and 27 components in positive ESI (Figure 7A) and negative ESI (Figure 7B), respectively), with 6 metabolites exhibiting significant differences from the sham group. Fatty acid PE 40:4, indolol, and lysophosphatidylethanolamine (LPE) 22:4 showed lower expression in the treatment group than in the sham group (P < 0.046, 0.034, and 0.022, respectively). Conversely, 1-benzoyl-3-phenylpropyne, salidroside, and sarcostin displayed higher relative abundance in the treatment group than in the sham group (P < 0.08, 0.49, and 0.05, respectively).

Discussion

The polyherbal Wattana formula (WNF) has been employed for health promotion, prevention of aging-related conditions, and gastrointestinal symptom treatment for over 3 decades [1]. Investigating the efficacy and metabolomics of this medicine may enhance its practical applications. This study examined the impact of WNF on ileum contraction, intestinal transit, food intake, and body weight under various conditions in an animal model.

Prior studies on WNF components have demonstrated significant reductions in smooth muscle contraction through multiple mechanisms [16–18]. In this investigation, which used isolated rat ileum, ACh was employed as a standard drug to induce smooth muscle contractions via muscarinic receptor activation.

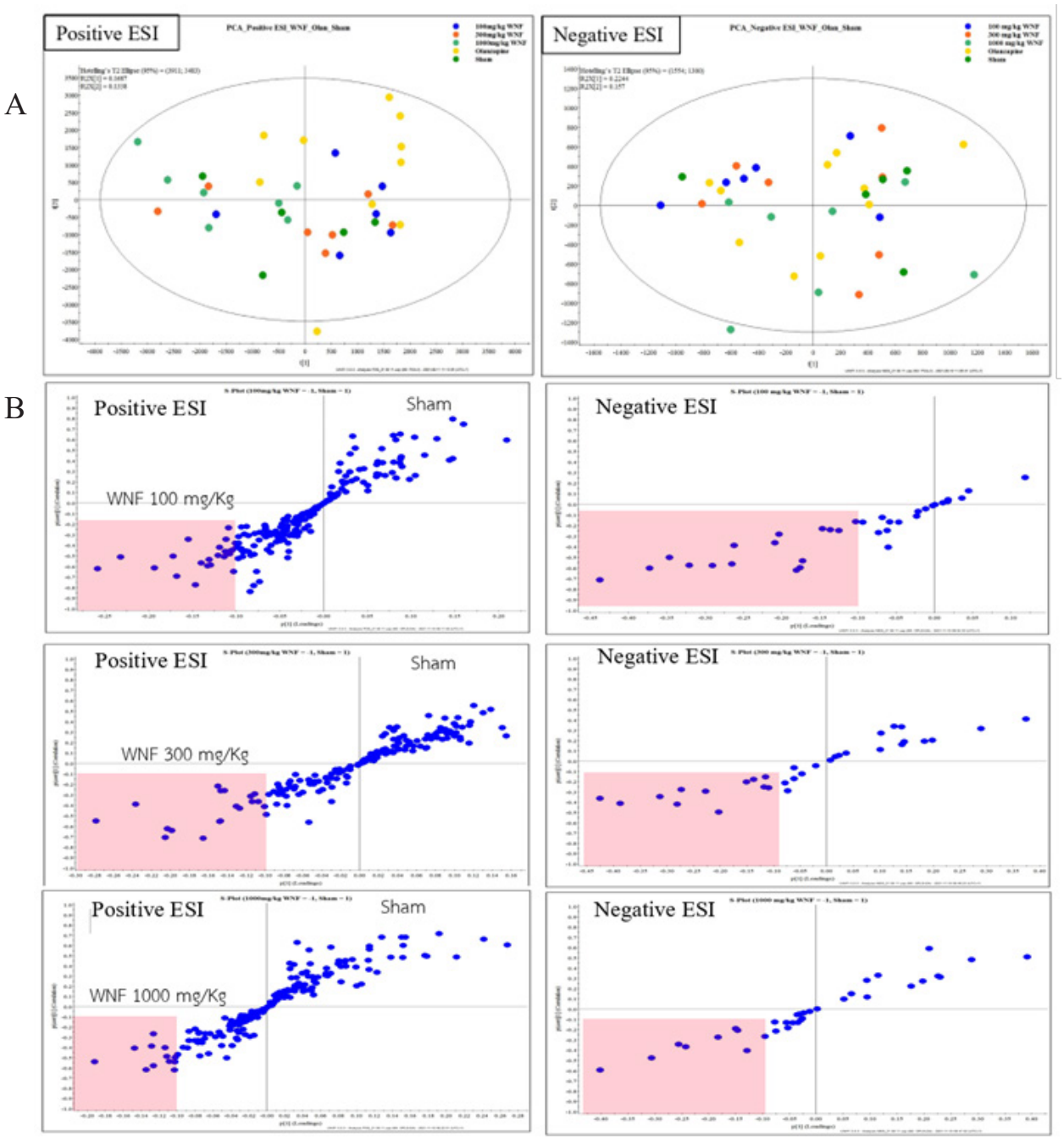

Figure 6. PCA plot and S-plot of Wistar rat metabolomics following WNF, vehicle, and olanzapine administration.
(A) Positive and negative ESI PCA plots display the reliability of group differences. Green = sham group; blue = 100 mg/kg WNF group; orange = 300 mg/kg WNF group; light green = 1000 mg/kg WNF group; and yellow = olanzapine group. (B) The S-plot reveals reliability for separation between the sham and WNF administration groups (100 mg/kg, 300 mg/kg, or 1000 mg/kg). The OPLS-DA S-plot of each variable employs cutoff values for the covariance of $P \geq |0.05|$ (magnitude) and $p(corr) \geq |0.1|$ (reliability), indicating the most distinct compounds for each group.

Atropine, a muscarinic receptor antagonist, can entirely inhibit ACh-induced contractions [19,20] and decrease the propulsive movement of charcoal meals due to its anticholinergic effect [12,21,22]. This study reveals that 600 µg/mL WNF inhibits ACh-induced contractions in isolated rat ileum similarly to atropine, suggesting its potential inhibitory effect on the muscarinic receptor.

The potential effects of WNF are demonstrated by this study's observation that 1000 mg/kg WNF significantly decreased the intestinal transit distance of charcoal meal compared to the vehicle group. Components such as *Cyperus rotundus* L., *Piper nigrum* L. and *Aucklandia lappa* (Decne.) Decne. have been reported to inhibit intestinal transit. Additionally, spicy herbs in WNF, such

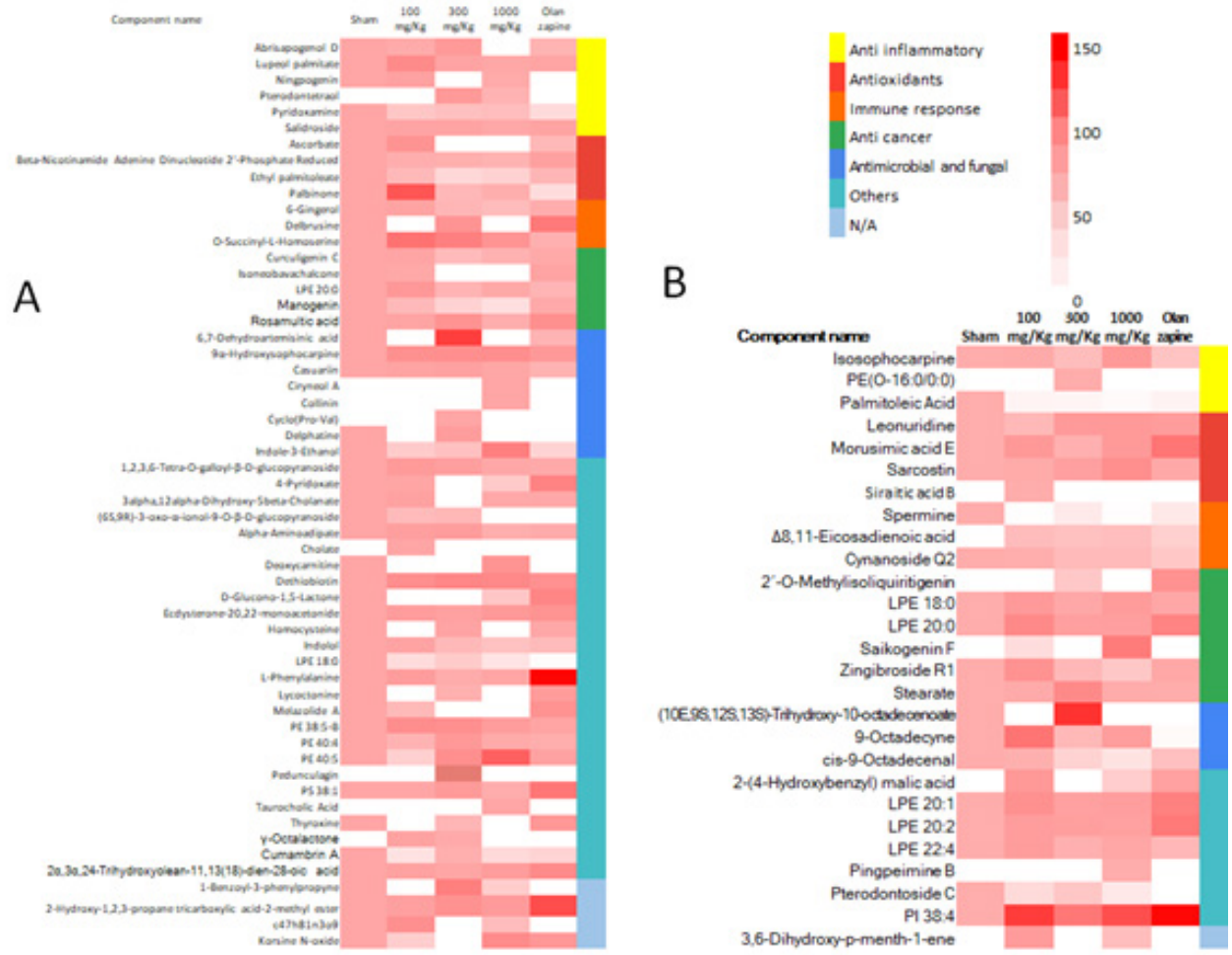

Figure 7. Untargeted metabolomics profiling of Wistar rats following WNF administration. This figure displays positive ESI (A) and negative ESI (B) plots with respective heatmaps for 83 metabolites across treatment groups. (A) In positive ESI, the heatmap identifies 56 metabolites, with 22 showing decreased levels and 34 showing increased levels in the WNF-administered group compared to the sham group. (B) In negative ESI, the heatmap identifies 27 metabolites differentiating treatment groups, with 11 exhibiting decreased levels and 16 showing increased levels in WNF administrations compared to the sham group.

as *Cinnamomum ilicioides* A.Chev., *Cladogynos orientalis* Zipp., *Cyperus rotundus* L., *Ferula assa-foetida* L., and *Piper nigrum* L. [1,23], can inhibit gastrointestinal transit in rats and mice [24] while enhancing lipid metabolism [25]. Spicy herbs may also offer obesity-related benefits through capsaicin [26], chili's major active principle [27]. Capsaicin, a transient receptor potential vanilloid type 1 (TRPV1) agonist [26], can reduce body weight and adipose tissue, and attenuate glucose intolerance, fat accumulation, inflammation, and insulin resistance [26]. Piperine, an active principle of pepper, exhibits antisecretory effects on mouse intestines [28] and inhibits gastrointestinal transit in rats and mice [24]. Moreover, *Cladogynos orientalis* Zipp., *Cyperus rotundus* L., and *Piper nigrum* L. can induce sleep [1, 29,30], which may profoundly inhibit gastrointestinal motility [31,32] and delay gastric emptying rates [33]. These findings imply that WNF might offer additional clinical advantages in managing functional gastrointestinal disorders such as intestinal hypermobility due to diarrhea.

Olanzapine, an atypical antipsychotic, has been demonstrated to increase weight and appetite in human studies [34-36]. In contrast, numerous animal studies have shown olanzapine to increase food intake without inducing weight gain in male rats [37,38]. In the current investigation, olanzapine increased both food intake and weight gain. Compared to the vehicle group, 1000 mg/kg WNF significantly reduced the weight gain rate without affecting food intake. Five WNF components, namely *Piper nigrum* L., *Aegle marmelos* (L.) Corrêa, *Boesenbergia pandurata* Schltr., *Carthamus tinctorius* L., and *Tinospora crispa* (L.) Hook.f. & Thomson, have been reported to enhance metabolism and reduce body weight [25,39-42]. The observed decrease in weight gain rate after WNF consumption might be attributed to metabolism enhancement, as gallic acid, a major active principle in WNF [2], has been shown to promote weight loss and reduce adipose tissue weight in rats [3]. These findings suggest that

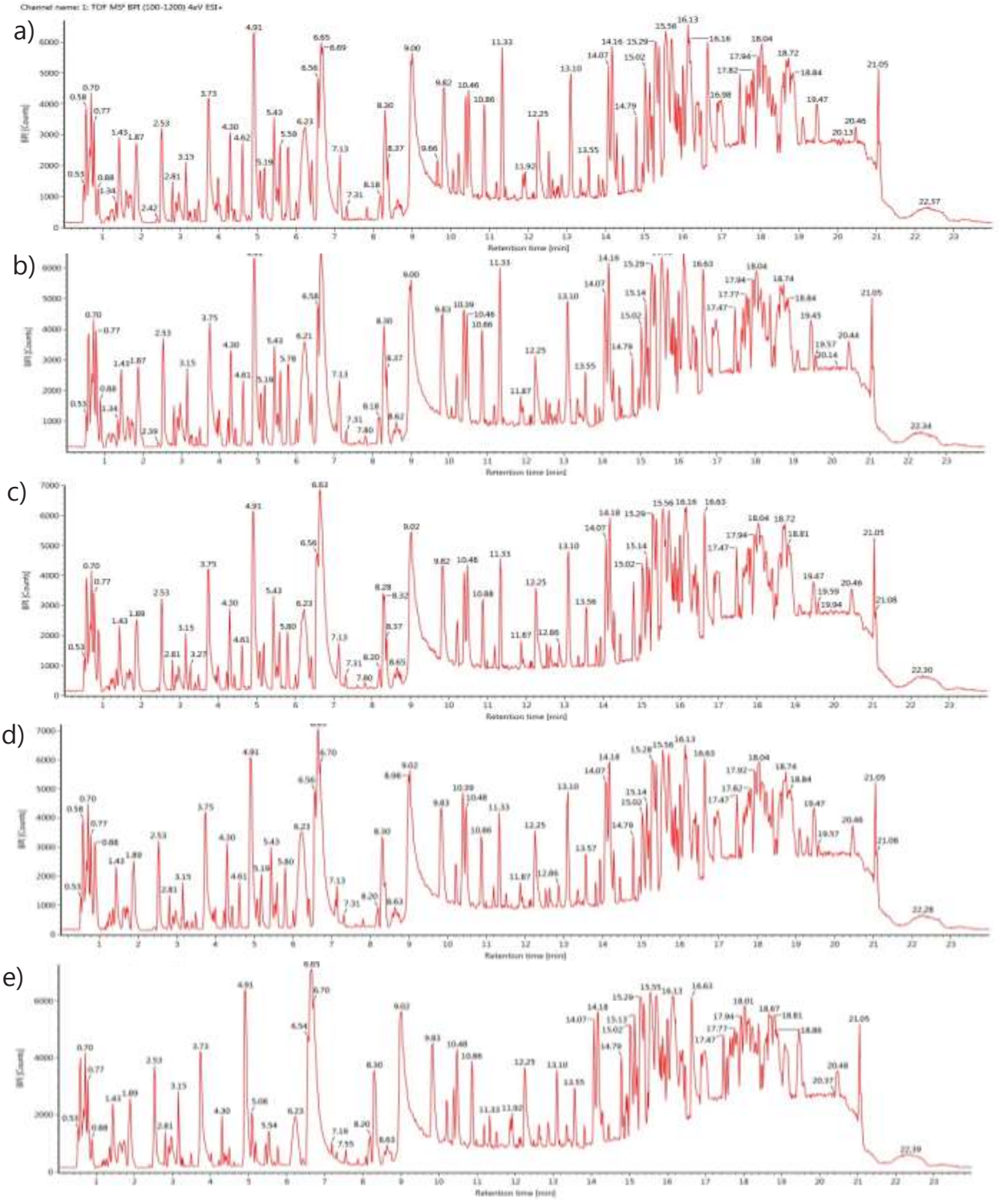

Figure 8. Chromatography of metabolomics profiling study in positive ESI. The chromatography of metabolomics profiling study divided into 5 groups; a**)** Sham, b**)** 100 mg/kg WNF, c**)** 300 mg/kg WNF, d**)** 1000 mg/kg WNF and e**)** Olanzapine**.**

WNF may be beneficial for obese patients.

Untargeted metabolomics analysis revealed numerous metabolites in the WNF-administered group compared to the vehicle group. A total of 83 metabolites were identified in the Wistar rats, with 6 metabolites being exclusive to the experimental group. Fatty acid PE 40:4, indolol, and LPE 22:4 exhibited lower expression in the treatment group, while 1-benzoyl-3-phenylpropyne, salidroside, and sarcostin showed higher relative abundance compared to the sham group. Fatty acid PE 40:4 is one of the eight phospholipids associated with noncommunicable diseases especially hypertension and obesity. The PE

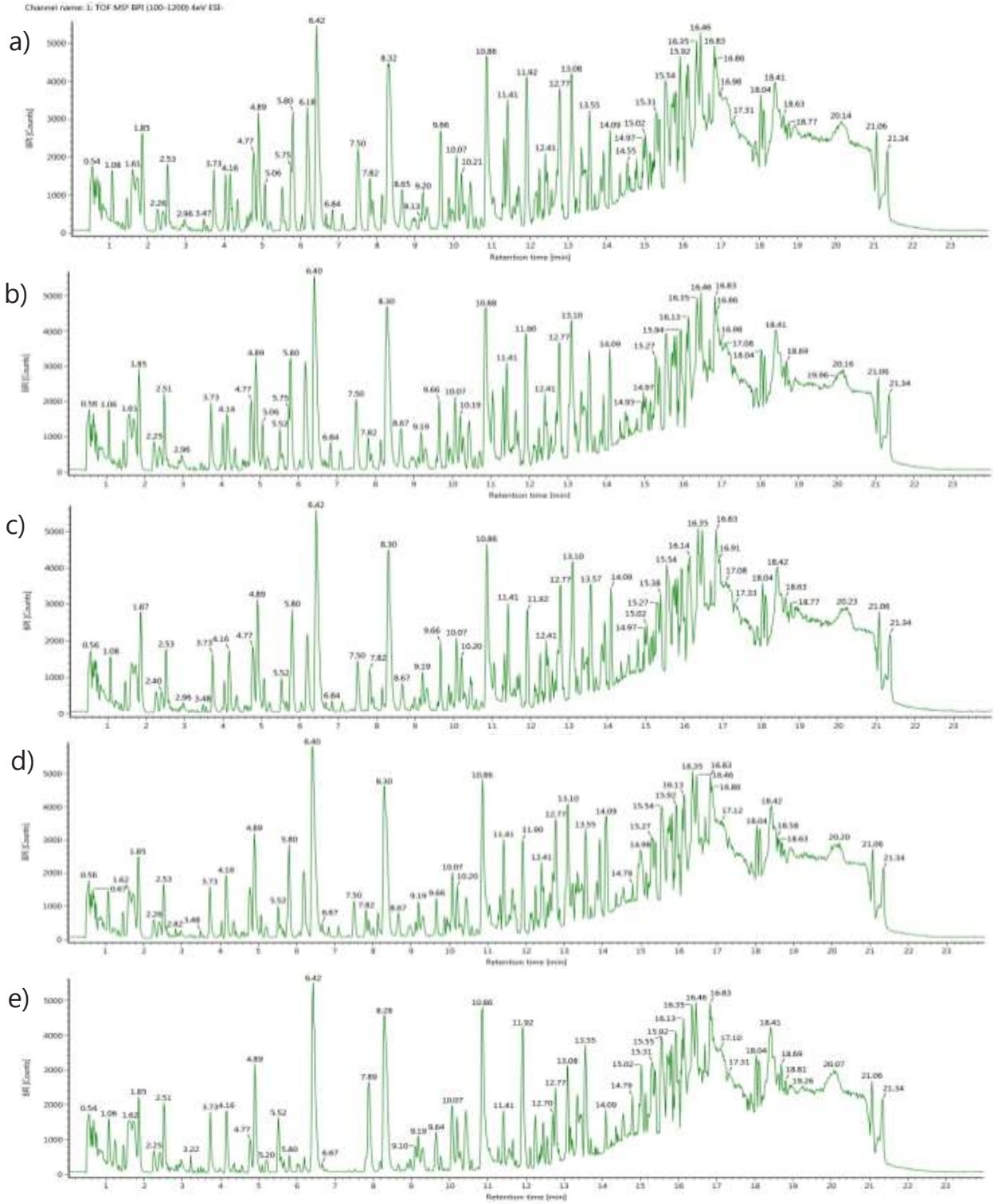

Figure 9. Chromatography of metabolomics profiling study in negative ESI. The chromatography of metabolomics profiling study divided into 5 groups; a**)** Sham, b**)** 100 mg/kg WNF, c**)** 300 mg/kg WNF, d**)** 1000 mg/kg WNF, and e**)** Olanzapine. Red= Positive ESI and green = Negative ESI

40:4 showed the improved the discrimination model of incident hypertension. Strong associations were identified of these phospholipids with triglycerides with obesity related traits [43]. The result showed the decrease of PE 40:4 after WNF administration. These findings probably suggest a potential role for WNF in helping to improve health promotion about the obesity disease. LPE 22:4 is a lipid class associated with gastrointestinal tract inflammation. A study compared plasma lipid profiles before and after Exclusive Enteral Nutrition (EEN) in adults

with active Crohn's disease to those of healthy controls [44]. The findings revealed a significant decrease in LPE levels after EEN which is according to the result of the treatment group with WNF. These results suggest that WNF might aid in reducing inflammation in the digestive tract. Indolol, 1-benzoyl-3-phenylpropyne, salidroside, and sarcostin currently lack direct report for use specifically related to the gastrointestinal tract. Among the 83 metabolites, pterodontoside C and 2α,3α,24-trihydroxyolean-11,13(18)-dien-28-oic acid had not been reported in prior WNF-administration literature. Pterodontoside C belongs to the terpene glycoside group, while 2α,3α,24-trihydroxyolean-11,13(18)-dien-28-oic acid is part of the triterpenoid group and is found in the *Terminalia* genus of the Combretaceae family. *Terminalia chebula* Retz. (also known as myrobalan wood and Samor Thai) has astringent and sour properties that can act as a mild laxative. Thai traditional medicine uses myrobalan wood as a laxative to facilitate the movement of the wind element and regulate bowel movements [45-47].

According to Samutthanwinitchai scripture, pathogenesis diagnosis suggests that illnesses in humans over the age of 30 are dominated by the wind-body element (Dhatu Chao-Ruean). Such illnesses are frequently diagnosed and are considered more serious than sicknesses caused by other body elements [42]. WNF's hot taste (RoT Prathan) helps distribute the wind element, making it ideal for treating adult illnesses and maintaining health. The therapeutic properties of WNF include a carminative effect, appetite stimulation, and muscle pain reduction. *Boesenbergia rotunda* (L.) Mansf., *Ferula assa-foetida* L., and *Piper nigrum* L. are the primary ingredients for carminative [45,48,49], antispasmodic activity [48], and promoting digestion [45]. The other ingredients support these primary ingredients to balance the 4 elements and restore health. *Aucklandia lappa* (Decne.) Decne., *Ligusticum sinense* Oliv., and *Cyperus rotundus* L. have anti-inflammatory and antispasmodic effects on the intestine [50-53] and an antibacterial effect on diarrhea [53]. In addition to its antipyretic [48] and antispasmodic activity [54], *Tinospora crispa* (L.) Hook.f. & Thomson has a cool-bitter taste that helps to maintain body temperature and stimulate appetite.

Given the small sample size, additional research is necessary to draw a conclusive inference. Moreover, a targeted metabolomics study utilizing elemental compositions could confirm the potential metabolites identified post-WNF administration.

In summary, this study suggests that WNF may decrease body weight, intestinal transit, and isolated rat ileum contractions without affecting food intake and without showing toxicity. Clinical trials are needed to further investigate the clinical outcomes of WNF. WNF shows potential as an agent in weight reduction, intestinal transit inhibition, and smooth muscle relaxation.

Conflict of Interests

None.

Acknowledgments

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