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Original Article

Identification by Docking Simulation and *in vivo* Effect of Essential Oil from *Cinnamommum burmannii* as Anti-obesity with Leptin Receptor in the Olfactory System of Mice Balb C

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ABSTRACT

Aim: This study examines the effect of inhalation of essential oil of cinnamon (*Cinnamomum burmannii*) on the metabolic activity of hormone receptors olfactory system of mice Balb C. **Methodology:** Effects of agonist or antagonist compounds in cinnamon essential oil on metabolic hormone receptors in the olfactory system are predicted using molecular docking simulation. Changes in the metabolic processes that occur views of changes in body weight, change in food intake, as well as lipid profile and blood glucose of mice. **Result:** The results showed Expression of leptin receptors (Lep-R) in the brains of mice given either inhalation of essential oils derived from the leaves and stems, in contrast to the control group who did not get essential oils. Provision of essential oils through inhalation increased lep-R expression in the brain of mice. Both *in silico* and *in vivo* evidence that essential oils from cinnamon plants are extracted from *Cinnamommum burmannii* and given by inhalation in Balb C mice are known to improve glucose and lipid metabolism by reducing the concentration of serum leptin concentrations and increased sensitivity to insulin.

Keywords: Olfactory system, Leptin receptors, *Cinnamomum burmannii*, Docking simulation, immunohistochemistry.

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INTRODUCTION

The general prevalence of adult obesity nationally in Indonesia is 19.1 % (8.8% obese and 10.3 % more obese), and the prevalence of central obesity to the national level is 18.8 %.[1] While data 2015 showed a general prevalence of adult obesity increased to 21.7 % which is a combination of the obesity. In children, the greatest prevalence of obesity was found in infants at 14 %, and the smallest at the age of 16-18 is equal to 1.4 %.[2] Obesity increases the risk for a number of serious comorbidities and often fatal as non-insulin dependent diabetes mellitus (NIDDM), dyslipidemia, cardiovascular disease, osteoarthritis, and certain types of cancers such as colon cancer and breast cancer post menopause.[3,4]

Obesity is a multifactorial condition. Such factors include genetic factors, diet, psychological states such as stress, physical activity, and factors related to the mechanism of the brain or the so-called cognitive factors and factors sensor.[5] Appetite initiated by smell (odor) works the olfactory system. Olfactory system is a sensor system (sensory) operating after the start of the transduction of chemical smells (odorants) to the occurrence of an action potential and limbic mechanisms that regulate and direct the behavior of a deliberate and need information of odor.[6] Smell is one of the primary senses that contribute to hedonic evaluation of food, thus affecting individuals in the selection and consumption of food. In the study in children aged 8-12 years with overweight shows that the trend patterns of overeating at the age of overstimulation triggered by their sense of smell by the smell of food compared to the factor-psychological factors such as mood, body esteem (assessment and acceptance of one's own body), and uncontrolled eating styles after chronic dietary/resist the urge to eat (restrained eating style).[7]

Compounds odorant (smell) work on the olfactory system. In the olfactory system, there are many receptors are associated with the metabolic hormones that play a role in regulating appetites such as insulin, leptin, and ghrelin. Currently, it is known that in addition to regulating appetite, hormones that work on the olfactory system also plays a role in the metabolism of nutrients. In the other study obtained by the fact that rats were given leptin - a hormone anorexigenic (appetite-suppressing hormone) - exogenous via intracerebroventricular (ICV) decreased body weight and

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food intake significantly within 48 hr.[8] Another study by Keung *et al* (2011) showed that administration of exogenous leptin via ICV in obese mice (mice were given a high-fat diet) then play to lose weight and food intake, it also served to increase fatty acid oxidation, and increased tolerance glukosa.[9]

In this research, immunohistochemical methods will be used to see on the metabolic activity of hormone receptors olfactory system Balb C after being given the essential oils from bark and leaves of the plant cinnamon (*Cinnamomum burmannii*) via inhalation. Metabolic hormone receptor activity in the olfactory system by the response observed secretion (a protein) that is produced due to the hormone-receptor binding in the hypothalamus. In the previous studies note that some essential oils are given to animals orally try to influence the process of metabolism (glucose and lipid profile) from rats/mice or rabbits induced diabetic stress oxidative.[11-13] Plant cinnamon is used as in previous studies note that the oil essential extracted from cinnamomi cortex and given orally in mice that are resistant to insulin is known to improve glucose and lipid metabolism by reducing the concentration of serum leptin concentrations and increased sensitivity to insulin.[14-15]

Effect of essential oils cortex in influencing metabolic processes has been empirically proven. However, what about the effect of essential oils via inhalation in influencing hormone-receptor binding in the olfactory system is not yet known. Though based on a study conducted by Peters *et al* (2007), Keung *et al* (2011), and Schulz *et al* (2012) note that the metabolic hormone (leptin) that works directly on the hypothalamus starts the process of interaction that occurs between the lep-R in system olfaktori.[8– 10] Given these facts , this study aims to look at the effect of inhalation of essential oil of cinnamon (*Cinnamomum burmannii*) on the metabolic activity of hormone receptors Balb C mice olfactory system by the protein that is secreted in the hypothalamus using immunohistochemistry method.

MATERIALS AND METHODS

Isolation of Essential Oils

Isolation of essential oils from botanicals cinnamon bark and leaves is done by using water distillation system Stahl. Simplicia dry powder of cinnamon bark and leaf inserted in a round pumpkin measuring 2 L. Next distilled water added with a ratio of 1:2 (w/v, g powder/µl distilled water). The distillation is carried out for 6 hr at temperatures ranging from 100-105ºC. Distillation produced a mixture of oil and distilled water. The two are separated after the mixture was placed in a separating funnel and allowed to stand for 24 hr. Separate oil included in the sample container to be used in the subsequent analysis phase.

Experimental animals

Adult male Balb C mice (25-30 g BB) were cultured in the laboratory of Experimental Pathology, Department of Anatomic Pathology Faculty of Medicine, was placed on the condition of 12 –hour light–dark cycle and given pellet diet and tap water *ad libitum*. All animal-related protocols will be asked to review the application of ethics to the Faculty of Medicine Ethics Committee. Provision of essential oils was based on the method carried out by Muctaridi *et al* (2011).[16] Mice were divided into 5 groups (each 5 shrimp) which consists of a control group, a group of stem bark essential oil (dose 0.2 and 1.0 µl /cage), and a group of leaf essential oil (dose 0.2 and 1.0 µl / cage). Essential oils are given by way of placing mice in the indoor/enclosed cage measuring 40x40x30 cm^3 previously steamed with essential oils as much as 2 times daily for 15 min before feeding. The treatment period was for 21 days. Every 7, 14, and 21 days mice were taken for measurement of blood glucose and blood fats. After day 21, mice were sacrificed and dissected for examination of his brain taken hormone metabolic activity in the hypothalamus.

Measurement of body weight and food intake

Measurement of body weight of mice was measured every day 3 hr after lights on. The amount of food intake was calculated from food left over from feeding twice.

Measurement of blood glucose and lipid

Measurement of glucose and blood fats are carried out based on the method carried out by Keung *et al* (2011).[10] Blood glucose was measured after mice fasted for 16 hr using a glucometer. Blood fat (triglycerides) were measured as glycerol using the spectrophotometric method.

Measurement of the metabolic activity of hormone secretion response by hormone–receptor binding in the hypothalamus by immunohistochemistry

Brain tissue samples were fixed with phosphate–buffered formalin 10 % for 10 hr at 4°C, then hydrated in graded ethanol concentrations. Once passed in xylol, made paraffin blocks. Hypothalamic brain tissue sections cut at a thickness of 4 µm for immunohistochemistry staining. Once done deparaffination and rehydration, preparations dyed in 0.01 M citrate buffer (pH6.0) in a microwave for 5 min. Etched with dosage 3 % hydrogen peroxide to eliminate endogenous peroxide for 5 min at room temperature. Preparations were incubated with antibody of the target protein (a protein produced in response to metabolic hormone secretion – receptor) in PBS for 2 hr at room temperature in a humidity chamber. Further, preparations were incubated overnight at 4°C. Used as a negative control N – Universal negative control (Dako). Preparations were then incubated with the appropriate secondary antibody for 1 hour at room temperature, followed by incubation for 30 min with HRP–conjugated streptavidin. Protein visualized using 3, 3'– diaminobenzidine (DAB) for 10 min at room temperature. The preparation was added counterstain with Harris Hematoxylin, hydrated and done the mounting.

Molecular Docking

The process begins with the preparation docking file is done using the docking program contained in the software Autodock Vina. Both ligand and protein molecules, hydrogen were added to both polar and Gasteiger charge while the hydrogen nonpolar were merge. Files are stored in the ligand and the enzyme mole and pdbqt format for later use in the preparation parameters. Dimensional grid box used was 60 x 60 x 60 with a grid spacing of 0.375 Å. Docking calculation algorithm is run with parameters Lamarckian Genetic Algorithm (LGA) with a population size of 150, as many as 10 million energy evaluations and the repetitions (engine runs) as much as 100 times. These parameters are stored in the format. Pdbqt as a file that will be used to run the docking process.

RESULTS

Docking simulation

Simulation methods using *in silico* tools or software used in the initial screening of bioactive compounds for drug candidates. It is caused by the *in silico* approach, the interaction between the protein targets of bioactive compounds. To determine the level of interaction with the target bioactive compounds is done by using the approach of docking software AutoDock Vina. Docking as an initial screening process between the molecules of bioactive compounds that can bind to the active site Leptin. Initial screening based on the value of Gibbs energy, log P, inhibition constants, conformational structure, affinity, efficacy and the bonds between them are simulated. *In silico* simulation results as described in Table 1.

Table 1 : Lep-R docking with compounds in cinnamon

Ligands	ΔG	Pki	H don/acc	Hbond
Benzaldehyde	-7.8740	6.580	2	Lys 149, Pro 125
Sinamic acid	-5.6544	5.959	1	Val 161
Torreyol	-5.4563	4.835	1	Glu 48

Olfactory receptor system targets obtained from the PDB (Protein Data Bank) with PDB code (3V6O). Volatile compounds in cinnamon after extracted and found 30 volatile compounds were analyzed Lep-R docking compounds in cinnamon. Docking process will produce three outputs which can be analyzed further, first the orientation and position of the resulting ligand as an inhibitor of the receptor. The second one can identify compounds that have an affinity for the protein of database compounds available and the third is to predict whether a molecule has an affinity for the receptor. Docking calculation results were seen in the output in the format viewer.mdb.

As shown in Table 1, compound benzaldehyde, cinnamic acid, and torreyol have ΔG values lower than other compounds of the lep-R. These results indicate that the compound has the above ligand complex conformational stability higher than others. It is confirmed that all three compounds have affinity better than others volatile compounds.

In vivo Test of volatile oil from *Cinnamomum burmannii*

Effect of cinnamon essential oil inhalation on body weight of mice.

Results of cinnamon essential oil inhalation on mice weight every week where week 1 is the weight of mice before inhalation, for each group is the control group, the group of essential oils from cinnamon sticks with doses of 20 and 40 µl of leaf and cinnamon with a dose 20 and 40 µl is shown in Figure 1 below.

In the Figure 3 can be seen that happen in all groups of mice, including weight gain in the control group from baseline to the end of the study. Highest weight of mice possessed by the control group. The group with a lower weight in order are granting group essential oils derived from the stem with a dose of 40µl and leaves with 20µl dose.

According to the results of statistical tests using two-way analysis of variance, the results obtained are significant differences in the groups tested (p = 0,00). At the smallest difference test using Duncan's test, showed that the control group and the group of essential oils 20µl stem have a higher weight than the group of essential oils 40µl stems, 20 and 40µl leaves. While the provision of essential oils 40 µl stems, leaves 40 µl and 20 µl on the test showed no significant difference. Of two-way analysis of variance test also indicated that there was no difference in body weight of mice in each week conducted the study (p=0.608). Of these tests also indicated no interaction between group and week in influencing the mice body (p=1.000).

Effects of inhalation of essential oils of cinnamon on blood glucose levels of mice

Result of inhalation of essential oils of cinnamon on blood glucose levels of mice every week where week 1 is the weight of mice before inhalation, for each group is the control group, the group of essential oils from cinnamon with doses of 20 and 40 µl from stems and leaves is shown in Figure 2 below. In the Figure shows that in all groups there was an increase in blood glucose levels in the control group of mice, including research from the beginning until the end of the study, although the increase is not constant. Blood glucose levels are highest mice belong to the group of mice with the provision of essential oils derived from the leaves with a dose of 20µl. Other groups who have lower blood glucose levels are a group of essential oils derived from the provision of the stems with doses of 20 and 40µl, 40µl leaves with a dose of the control group.

According to the results of statistical tests using two-way analysis of variance, the results obtained are significant differences in the groups tested (p=0.00). At the smallest difference test using Duncan test, it appears that the control group had a different blood sugar levels and significantly lower than the stems with dose 20 and 40µl, and leaves with dose 20 and 40µl. While the provision of stem essential oils with dose 20 and 40µl and leaves essential oils with dose 20 and 40µl on the test showed no significant difference. Of two-way analysis of variance test also indicated that there are differences in blood glucose levels of mice in each week doing research (p=0.00). Of these tests also indicated no interaction between group and week affect blood glucose levels in mice (p=0.382).

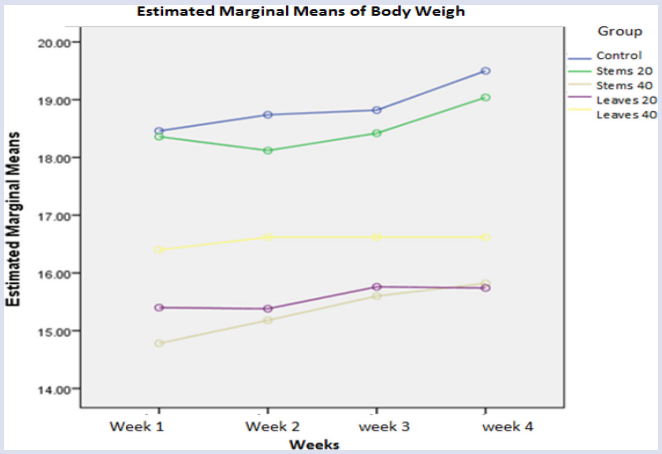

Figure 1 : All groups of mice body weight giving essential oil (derived from the stems and leaves of 20µl and 40µl) from the beginning to the end of the study are expressed in grams.

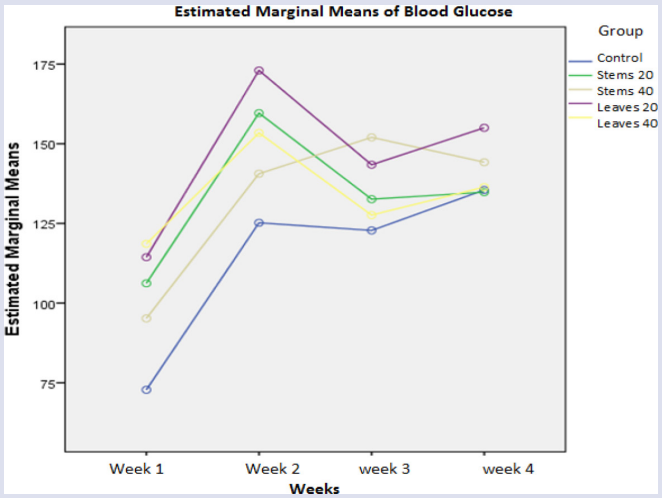

Figure 2 : Blood glucose levels of mice all groups providing essential oil (derived from the stems and leaves of 20µl and 40µl) from the beginning to the end of the study were expressed in mg / dL

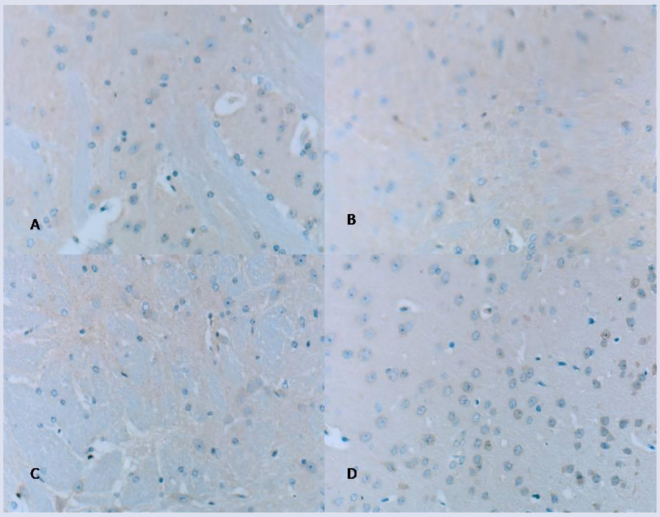

Figure 3 : Staining of immunohistochemical of lep-R in the leaf essential oil doses of 20 µl (A) with negative expression, essential oils leaves 20 µl (B) positive 1, and stems 40 µl (C) 2 positive, and leaves 40 µl (D) positive 3. Observations were carried out using a light microscope with 400x magnification.

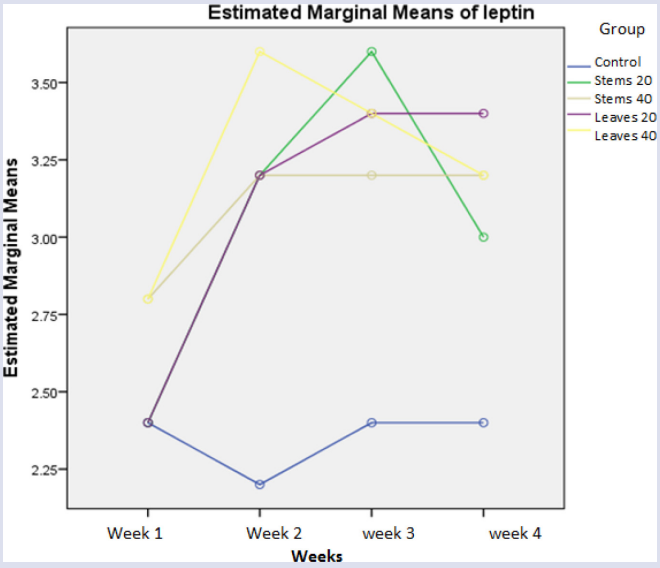

Figure 4 : Expression of lep-R in all groups of mice brain tissue was observed using immunohistochemical methods.

Lep-R expression in Mice Brain Network

Examination of the expression of the lep-R (Lep–R) in the brain tissue of mice performed using immunohistochemical methods (Figure 3). Ten results on visual field examination were made scores positivity receptor expression as assessed by two researchers (double observer). Positivity declared negative if the ten field of view there is no brain cells at all express hormone receptors. Tested positive for 1, if the field of view of 0-2 cells is positive, tested positive for 2, if the field of view of 3-5 cells is positive. 3 positive if 5-10, 4 positive if more than ten cells per field of view. Immunohistochemical staining results shown in the following Figure 4.

In Figure 4 above shows that the lep-R (Lep–R) as a whole experienced the differences between all treatment groups with the control group. It appears that the control group had an expression of Lep–R is lower than all group. Whereas among treatment groups, from the graph above does not show the real expression differences. This was reinforced by the statistical test using the Kruskal–Wallis test showed that there are at least one different treatment groups. Through the Mann-Whitney test indicated that the difference between the control group with the other groups. Found no difference between the control group than the other groups.

DISCUSSION

Effects of agonist or antagonist compounds in cinnamon essential oil on metabolic hormone receptors in the olfactory system are predicted using molecular simulations. Simulation molecules are also used to predict the hormones involved or affected by the content of the compound in cinnamon. Based on the data generated can also be specified target protein response result secretion of the hormone–receptor interactions to be tested by immunohistochemistry.

As shown in Figure 5, compound benzaldehyde able to enter the binding of lep-R and alter conformation. Moreover, compared with the other compounds, compounds benzaldehyde have more hydrogen bonding interactions on lep-R, so it can be said that the compound has the ability benzaldehydee as a lep-R inisiator.

As Figure 6, the more the hydrogen bonding complex stability and the ability of the stronger inhibition of the active compounds is higher. From Figure 6 looks even further, docking simulation analysis showed that the two compounds that have the amount of hydrogen donor and hydrogen acceptor more than the other compounds. This indicates that the two compounds have a hydrogen bonding interaction is stronger in the lep-R catalytic than other compounds.

Based on molecular docking results of this assessment, it is possible and reasonable that the compound Benzaldehyde and cinnamic acid is

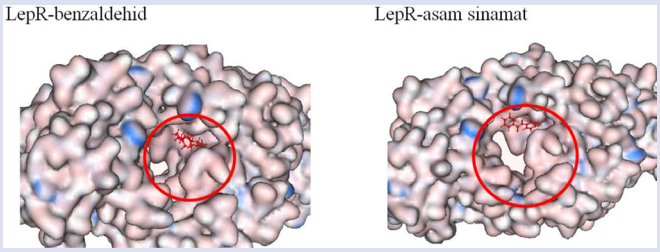

Figure 5 : Complex of LepR-ligands (Benzaldehyde and cinnamic acid) in catalytic side

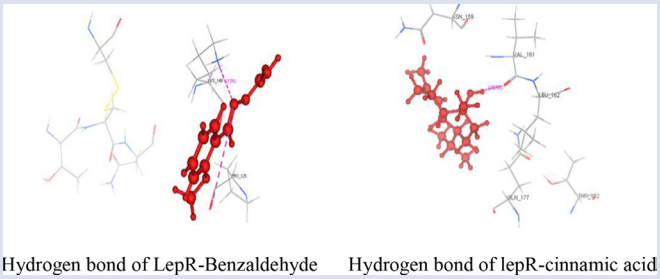

Figure 6 : Hydrogen bond to the lep-R Benzaldehyde compounds and cinnamic acid

able to hold a bond with Lep-R of the olfactory system, it is extremely grounded and is expected to be a candidate for therapy reduce obesity and reduce appetite in people with obesity.

Results of this *in vivo* study showed that inhalation of cinnamon essential oil such as leptin administration similar to another researcher that rats were given leptin – a hormone anorexigenic (appetite-suppressing hormone) – exogenous via intracerebroventricular (ICV) decreased body weight and food intake significantly within 48 hour.[8] Another study showed that administration of exogenous leptin via ICV in obese mice (mice were given a high-fat diet) then play to lose weight and food intake, also served to increase fatty acid oxidation and increased tolerance glucose.[9] The effect of weight loss and food intake also occurs in obese rats given exogenous leptin via intranasal.[10] Based on these studies it can be concluded that the effect of decreasing body weight and food intake by leptin beginning of leptin binding to its receptors on the olfactory system which then affects the activity of the hypothalamus in response to appetite and metabolism.

Lep-R expression in the brains of mice given either inhalation of essential oils derived from the leaves and stems, in contrast to the control group who did not get the essential oils. Provision of essential oils through inhalation increased lep-R expression in the brain of mice. Essential oils derived from the leaves and stems, either with a dose of 20 or 40 µl inhalation, no effects were different from each other.

CONCLUSION

Both *in silico* and *in vivo* evidence that essential oils from cinnamon plants are extracted from *Cinnamommum burmannii* and given by inhalation in Balb C mice are known to improve glucose and lipid metabolism by reducing the concentration of serum leptin concentrations and increased sensitivity to insulin.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Lep-R: Leptin-Receptors; **NIDDM:** Non-Insulin Dependent Diabetes Mellitus; **ICV:** Intracerebroventricular; **LGA:** Lamarckian Genetic Algorithm.

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