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Research Article
Combination of *Andrographis panniculata* Ness, *Curcuma xanthoriza* Roxb and *Cinnamommum burmanii* as Appetite Enhancer with Low Infertility Risk

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Abstract

Background and Objectives: The main ingredients of Sambiloto (*Andrographis panniculata* Ness), Temulawak (*Curcuma xanthoriza* Roxb) and Kayu Manis (*Cinnamomum burmanii*) are andrografolid, curcumin and cinnamaldehyde, respectively. These compounds, in individual usage were reported as appetite enhancer in Wistar rats, but they caused infertility in male rats. This study therefore aimed to test the effectivity improvement of these compounds as appetite and fertility enhancer when administered as combination. **Materials and Methods:** A total of 36 male Wistar rats were divided into several groups including: treatment groups that were administered: Sambiloto-Temulawak-Kayu manis (STK), Sambiloto-Temulawak (ST), Sambiloto-Kayu manis (SK), Temulawak-Kayu (TK) manis, a positive control group was Tracetate treated and a group that was treated with vehicle. At the end of 28 days treatment, rats were sacrificed, testicle and spermatozoa were collected. The impairment of testicle tubules and reduction of primary spermatocyte amount were observed. **Results:** The body weights of rats were increased in all the treatment groups as compared to negative control group. Similar trend was observed in the percentage of food intake. Wistar rats in Sambiloto-Temulawak-Kayu manis (STK) group showed the lowest number of both spermatozoa viability of 41.33±4.01% (0.0004) and primary spermatocyte count of 43.70±6.91 cells (0.0044), but no impairment in seminiferous tubules was observed. **Conclusion:** The combination of sambiloto, temulawak and kayu manis (60:140:50 mg kg^{-1} b.wt.) increased body weight and food intake of male rats in this study. Sambiloto-Temulawak (ST) group shown as the best combination to increase body weight of rats with the lowest infertility effect.

Key words: *Andrographis panniculata* Ness, *Curcuma xanthoriza* Roxb, *Cinnamomum burmanii,* appetite enhancer, infertility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Appetite is a yearning to eat that can be due to hunger[1]. It is also defined as a natural desire to satisfy a bodily need, especially for food. Eating may provide the nutrition required by the body to create energy, to maintain health, growth and to make every organ functions properly. Therefore, a good balance between nutrition taken with the nutrition needed should be maintained. The balance between available nutrition and the body needs should be optimal to avoid over or lack of nutrition[2].

As we are aging, biological factors change the pattern of eating and appetite. Food intake tends to decrease even in a healthy elderly. This condition is known as "elderly anorexia aging", which commonly occurs in men rather than women[3]. This was supported by Wilson and Morley[4], which stated that the change of flavor in the sense of taste can affect appetite and the ability to enjoy food.

In order to find an alternative to improve appetite, we studied the combination of plants that are known for their ability in increasing appetite individually. Sambiloto (*Andrographis paniculata* Ness), Temulawak (*Curcuma xanthoriza* Roxb) and Kayu manis (*Cinnamomum burmanii*) are indigenous Indonesian plants known as appetite enhancer[5] that, respectively effective [6-8] at the dose of 60, 140 and 50 mg kg^{-1} b.wt. Interestingly, these plants cause infertility in male subject. Andrografolid in sambiloto disturbs spermatogenesis by preventing cell division therefore decreases spermatozoa production and acts as a cytotoxic substance towards seminiferous tubules[9]. Curcumin, a major compound of temulawak increases estrogen level in the blood. The increment in estrogen level causes negative feedback in hypothalamus and decreases the production of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH)[10]. Cinnamaldehyde, the main ingredient of cinnamon is suspected to be toxic to male reproduction[11] due to its ability to increase progesterone, but decrease the production of testosterone. Spermatogenesis in male requires LH and FSH. Therefore, a lack in LH and FSH reduces the spermatozoa production.

Hence, no study has been conducted to search the efficacy of these three combinations. This study therefore was conducted to demonstrate the effectivity of combining the extract of sambiloto, temulawak and kayu manis to improve appetite and fertility by observing the increment of body weight, food intake, spermatozoa viability, impairment of testicle tubules and primary spermatocyte count in male Wistar rats.

MATERIALS AND METHODS

Animals: Total 36 male Wistar rats of 2 old months, 150-200 g were used in this study. This research project was carried out at Clinical and Community Department, Diagnostic Clinic Laboratory, Indonesia from April, 2014-2015. Prior to the experiment, rats underwent acclimatization to the new environment. During that period, rats were placed in free air circulation cage, supplied with *ad libitum* food and water.

Preparation of ethanol extract of plants: Fresh leaves of Sambiloto, rhizomes of temulawak and barks of kayu manis were purchased from Materia Medika, Batu, East Java Indonesia. The plants ethanol extracts was obtained by macerating those plants in 96% of ethanol separately. In order to produce a thorough extraction of the active compounds, 6 h stirring process was performed prior to an overnight extraction. Following filtration, the filtrate was collected and re-macerated in ethanol 96%. Liquid of the second filtration was evaporated until thick extract was yielded. Thin Layer Chromatography was then conducted to identify the presence of main component of each plant's extracts by using different mobile phases[12].

Experimental design: Thirty six Wistar rats were divided into 6 groups of 5 rats, namely: STK group (combination of Sambiloto Temulawak Kayu manis extract), ST group (extract combination of Sambiloto Temulawak), SK group (extract combination of sambiloto-cinnamon), TK treatment group (extract combination of temulawak-cinnamon), negative control group (CMC Na:PGA (1:1, 25) and positive control group (Tracetat 72 mg kg^{-1} b.wt.). The animals in each group were treated for 28 days[9]. Body weight was measured every other week. The amount of the consumed and remaining food was weighted every other day. After 28 days, rats were sacrificed and testicles were collected to observe the primary spermatozoid and tubular impairments. Spermatozoa fluid was taken and tested for their viability[12,13]. This study was approved by the Ethical Commission of Medical Faculty, Gadjah Mada University, Yogyakarta, Central Java, Indonesia.

Spermatozoa collection and observation of spermatozoa viability: Spermatozoa was taken from cauda of epididymis by pinching the epididymis edge and then pressed until homogenous suspension of spermatozoa was obtained[9]. Spermatozoa were mixed with 0.9% of NaCl in a watch glass and then it was dropped to object glass then stained by eosin to be observed under microscope in 400 times magnification.

Observation of impaired seminiferous tubules: Impaired seminiferous tubules was observed after the histology preparation was ready. It was conducted under microscope in 100 times magnification in five different fields of view for each preparation. Impaired tubulus showed less spermatogenic cell in order and lysis was seen inside tubules[9].

Primary spermatocyte counting: Primary spermatocyte was counted from seminiferous tubules histological slide under microscope in 400 times magnification. Primary spermatocyte is defined as a vast round shape, dark nucleus with distinct chromosome[14].

Statistical analysis: Statistical analysis was conducted by SPSS 17.0. The data presented as mean±standard error of means (SEM). Statistical significance was tested by one way ANOVA by considering the $p<0.05$ to be statistically significant.

RESULTS

Phytochemical screening: The chromatogram of Thin Layer Chromatography (TLC) identified the retardation factor of andrografolid (Rf 0.40), curcumin (Rf 0.61) and cinnamaldehyde (Rf 0.47). Observation was performed under UV 254 nm (Fig. 1). Table 1 displayed the phytochemistry contents of these plants extracts. The flavonoid was found in all the plants extract, while steroid was found in sambiloto.

Body weight: The increased of rat's body weight after the administration of plants extract combination were studied. This study reported no alteration of body weight compared to positive control group of 41.20±23.80 g, but significantly different to negative control group approximately 6.90±19.42 g. The SK group stimulated the highest increase of rat's body weight of 41.13±9.82 g (Table 2).

Table 1: Phytochemistry screening of sambiloto, temulawak and kayu manis extract

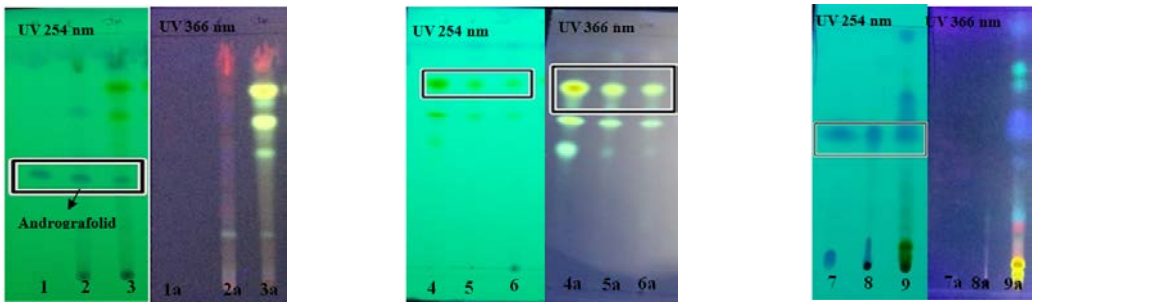
Parameters	Alkaloid	Flavonoid	Quinnon	Tannin	Saponin	Steroid	Terpene
Sambiloto	-	+	+	+	-	+	-
Temulawak	+	+	-	-	+	-	+
Cinnamon	+	+	-	+	-	-	+

+: Present, -: Absent

Table 2: Mean value of different parameters of sambiloto, temulawak and kayu manis

Treatment groups (%)	STK	ST	SK	TK	C(+)	C(-)
Body weight during treatment	34.40±19.21^a	34.90±12.48^a	41.13±9.82^a	34.90±24.80^a	41.20±23.80^a	6.90±19.42^b
Food intake during treatment	17.10±2.90^a	23.53±1.03^a	25.89±0.63^a	29.63±0.27^a	30.80±2.44^a	14.40±3.31^b
Spermatozoa viability	41.33±4.01^b	58.53±3.52^a	59.71±0.66^a	58.15±4.54^a	44.43±3.30^b	60.79±2.58^a
Seminiferous tubules impairment	75.04±10.35^a	73.47±7.19^a	58.47±12.46^a	72.59±2.32^a	92.66±4.84^b	73.47±3.05^a
Primary spermatocyte	43.70±6.91^b	50.03±4.92^a	50.57±1.91^a	50.95±1.58^a	42.32±5.50^b	55.07±2.30^a

Superscript of different letters showed significant difference between treatments, C: Control group, STK: Sambiloto temulawak kayu manis, SK: Sambiloto kayu manis, ST: Sambiloto temulawak, TK: Temulawak kayu manis



1. Andrografolid (5 µL)
2. Sambiloto extract (10 mg mL^{-1})
3. Combination extract (10 mg mL^{-1})
   Mobile phase:
   Chloroform: Methanol (9:1)

4. Curcumin (5 mg mL^{-1})
5. Sambiloto extract (1 mg mL^{-1})
6. Combination extract (1 mg mL^{-1})
   Mobile phase:
   Chloroform: ethanol: glacial acetic acid (9:1)

7. Sinamaldehyde (1%)
8. Sambiloto extract (10%)
9. Combination extract (10%)
   Mobile phase: toluene: acetic acid (97:3)

Fig. 1: Identification result using TLC at UV 254 nm and UV 366 nm

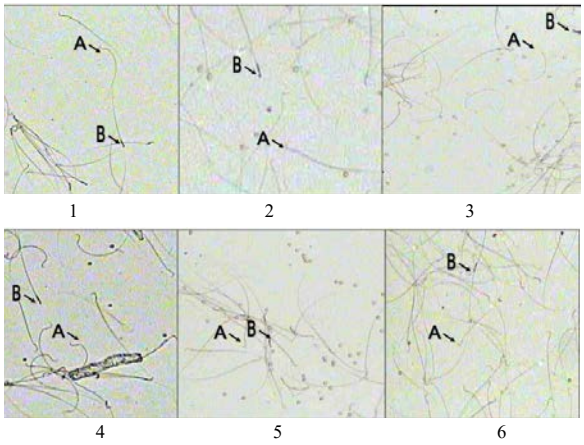


Fig. 2: Spermatozoa viability observation magnified 42.3×10
A: Live spermatozoa, B: Died spermatozoa, 1: Sambiloto temulawak kayu manis group, 2: Sambiloto temulawak group, 3: Sambiloto kayu manis group, 4: Temulawak kayu manis group, 5: Negative control group, 6: Positive control group

Food intake: The food intake was measured in treatment groups and found that male Wistar rats in treatment groups consumed food in similar amount with positive control group of 30.8±2.44 g, however negative control group consumed 50% lower amount of food of 14.4±3.31 g (Table 2).

Percentage of spermatozoa viability: The stained-viable spermatozoa were counted under microscope. It was observed that significant decrease of spermatozoa viability in STK group of 41.33±4.01% compared to negative control group (60.79±2.58%), but not in the other treatment groups (Table 2).

Impaired seminiferous tubules: The impaired seminiferous tubules were characterized by less ordered form of spermatogenic cell, the presence of multinucleic cell and unorganized form of lamina basalis (Fig. 3). It found that the increment of impaired seminiferous tubules was not different between treatment groups and negative control group, as compared to positive control group (Table 2).

Primary spermatocyte number: The primary spermatocyte is the vast-round shape cell with dark nucleus (Fig. 4). It observed that the lowest number of primary spermatocytes was induced by STK group of 43.70±6.91 cells which was similar to positive control group (42.32±5.50 cells). However, the other treatment groups showed the same primary spermatocyte number as negative control group (Table 2).

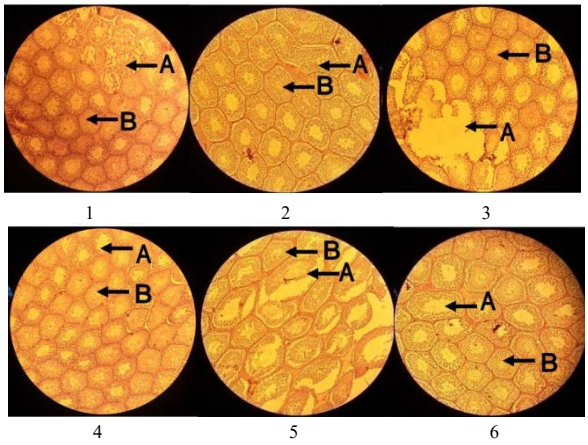


Fig. 3: Observation of seminiferous tubules impairment, magnified 10×10
A: Impaired seminiferous tubules, B: Good condition seminiferous tubules, 1: Sambiloto temulawak kayu manis group, 2: Sambiloto temulawak group, 3: Sambiloto kayu manis group, 4: Temulawak kayu manis group, 5: Negative control group, 6: Positive control group

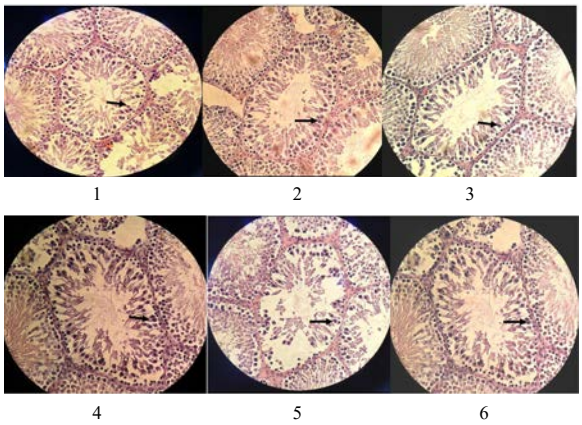


Fig. 4: Observation of primary spermatocyte, magnification 10×10
Arrow pointed the primary spermatocyte, 1: Sambiloto temulawak kayu manis group, 2: Sambiloto temulawak group, 3: Sambiloto kayu manis group, 4: Temulawak kayu manis group, 5: Negative control group, 6: Positive control group

DISCUSSION

In this study, it observed that an indication that the plants combination stimulated appetite and increased body weight. Results of current study showed consistency with the previous study of Andrografolid in sambiloto (*Andrographis paniculata*) that increased appetite of children[10] and in Wistar rats[12] by acting in the mucous membranes of the mouth and stomach[6]. Curcumin was found as the main compounds of temulawak (*Curcuma xanthorriza*) acts by faster emptying of stomach thus inducing appetite. In addition, Ciardi *et al.*[15] proved that

curcumin inhibits the release of leptin and satiety hormone in inflammation condition. In this way, curcumin is beneficial to induce the desire to eat more. Major compound of Kayu manis (*Cinnamomum burmannii*), cinnamaldehyde, were mostly found in the branch-bark and increases appetite by mechanism that cannot be scientifically explained yet.

Furthermore, a low number of primary spermatocyte count in STK group that was found to be different compared to negative control group. The decrement of primary spermatocyte amount in groups given sambiloto, temulawak and cinnamon extracts was speculated due to synergistic action of andrografolid, curcumin and cinnamaldehyde in plant extracts. Andrografolid in sambiloto affects spermatogenesis by preventing cytokinesis of dividing sperm cell lines thereby reducing sperm quality[9], while curcumin in temulawak works by inhibiting LH and FSH production[10]. Both LH and FSH are very important in spermatogenesis process. The LH stimulates Leydig cells in interstitial testicle to produce more testosterone while FSH stimulates Sertoli cells to produce androgen binding protein that are released into testicle fluid to bind testosterone.

In male, FSH is important in spermatogenesis process, as this hormone stimulates the process by initiating formation of both primary and secondary spermatocyte from spermatogonium. On the other hand, LH plays a role in testosterone secretion and spermatozoa initiation from spermatid. The FSH and testosterone work together in the Sertoli cell to produce various protein required by germinal cells to undergo proliferation, differentiation and cell metabolism thus maintaining normal spermatogenesis process[16,17].

The reduction of spermatozoa viability may occur due to less production of FSH, LH and testosterone. Those hormones increase sugar monomer secretion that is fructose by vesica seminalis, an accessory gland in male reproduction system. The fructose secretion by vesica seminalis provides nutrition, protects sperm and provides sources of energy for the ejaculated spermatozoa. In the diminished condition of fructose secretion, spermatozoa would not be able to obtain ample nutrition and energy to survive. In this way, spermatozoa is prone to death[18].

Similar to curcumin in temulawak and cinnamaldehyde in kayu manis was reported to decrease spermatozoa amount due to testosterone hormone decrement[11], In contrast to this report, Sariozkan *et al*.[19] mentioned that long term treatment of cinnamomum bark oil showed protective effect in sperm quality, testicular and epididymal oxidant/antioxidant balance, testicular apoptosis and sperm DNA integrity. Compounds of cinnamomum have been reported to decrease in testicular lipid peroxidation levels and increases in LH, FSH and testosterone[20] concentrations, reproductive organ weights, sperm count and motility, antioxidant activities (GSH, GSH-Px and CAT) and DST in healthy animals[21]. Furthermore, long term cinnamomum administration has been proven to protect testes, epididymides, accessory sex organs and spermatozoa and thus decrease testicular apoptosis against carbon-tetrachloride-induced reproductive toxicity by preventing oxidative stress[22].

This study implied that the combination of the 3 plant's extracts did not cause infertility, but stimulate increase body weight in male Wistar rats. Therefore this result can be applied to increase body weight of cattle with lower doubts of infertility issues. Since no combinational herbal study was conducted, this study however, suggested to assess the prolong usage of the combination to evaluate the infertility issues that might be seen. This study was lack of any other toxicity evaluation particularly in the liver and kidneys of the animal. This limitation might be utilized to direct a further elaboration of the plant extracts combination.

CONCLUSION

Conclusively, combination of sambiloto, temulawak and kayu manis at 60, 140 and 50 mg kg^{-1} b.wt., for 28 days is able to increase male Wistar body weight by stimulating appetite. On the other hand, the combination of these plants extracts did not stimulate infertility as observed from no alteration of spermatozoa viability, seminiferous tubules impairment and primary spermatocyte count in male Wistar rats.

SIGNIFICANCE STATEMENT

This study discovered the effectivity of herbs combination that can be beneficial for increasing appetite. People have been trying to feed cattle with herbs to increase appetite. However, some herbs show infertility effect. Therefore, we were looking for the best combination of herbs that increases appetite, without infertility effect by combining some well known plant extracts. To our knowledge, no combinational herbal study to increase appetite has been conducted so far. This study will help the researcher to uncover the critical areas of combining the best dosage of some herbs that many researchers were find it challenging to explore. Thus a new theory on formulating the most appropriate combination dosage may be arrived at.

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