Food Research 8 (Suppl. 2) : 87 - 101 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Finding the research gap of the potential anti-inflammatory activity of cocoa (*Theobroma cacao* L.) through systematic literature review

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Article history:
Received: 5 March 2023
Received in revised form: 27 September 2023
Accepted: 20 January 2024
Available Online: 6 April 2024

Keywords:
Anti-inflammatory effect,
Cocoa,
Flavanols,
Inflammation

DOI:
https://doi.org/10.26656/fr.2017.8(S2).60

Abstract

The use of conventional drugs in treating inflammation has various side effects, and thus it is necessary to have an alternative anti-inflammatory agent that is effective and safe. Cocoa (*Theobroma cacao* L.) is one of the agricultural products with a high content of polyphenols, especially flavan-3-ols potentially used as an anti-inflammatory agent. The purpose of this research is to investigate the research gap of the potential anti-inflammatory activity of cocoa based on the published literatures. The systematic method of synthesizing the review follows the guidelines of the PRISMA Statement 2020 using the PICOS literature search strategy. The results of this study showed that the positive effect of cocoa on inflammation indicated by 44 out of 72 biomarkers (61.11%). Flavan-3-ols can effectively reduce inflammation by reducing the activation of transcripts that regulate pro-inflammatory cytokines (NF-kB), prostaglandins, and NO. The anti-inflammatory effect was effectively found in different groups, i.e., subjects without health issue (healthy subject), subjects with some health problems record and subjects with obesity. Nevertheless, it was found in the previous researches that the manifest of cocoa-derived products as well as the biomarkers for determining the anti-inflammatory effect of cocoa still vary. Also, there is no study investigating the anti-inflammatory effect of cocoa on different age group. Therefore, further researches are required in this area to complete the puzzle of information regarding the potential anti-inflammatory activity of cocoa.

1. Introduction

Cocoa (Theobroma cacao L.) is of economic significance to some countries, including Indonesia (Praseptiangga *et al*., 2020). Nowadays, cocoa and its derived products are candidate of functional foods due to its functionality particularly for microbiota modulation (Zugravu and Otelea, 2019). Many researches have also shown that cocoa is naturally rich in polyphenols having beneficial effect on health (Fajardo *et al*., 2022). Major polyphenol in cocoa is flavonoid, including epicatechin, catechin and procyanidin (Carrillo *et al*., 2014). Some studies even attempted to improve the functionality of cocoa derived products including by adding herbs, spices, probiotics and other functional ingredients (Muhammad, Kongor and Dewettinck, 2021; Muhammad, Rahayu and Fibri, 2021; Muhammad, Tuenter, Patria *et al*., 2021; Muhammad, Zulfa, Purnomo *et al*., 2021). The enrichment of cocoa derived products with various functional ingredients is based on the fact that during the processing the polyphenols content of cocoa significantly decreases.

On the other hand, research interest in investigating anti-inflammatory effect of natural products have significantly increased in recent years. This is because inflammation may induce inflammatory disorders, and thus has a high association with cancer development (Muhammad and Dewettinck, 2017). The other possible risks of having inflammatory disorder are arthritis, alzheimer and atherosclerosis (Zuo *et al*., 2020). According to Zuo *et al*. (2020), there are two types of inflammation namely acute and chronic inflammation.

Inflammation can be treated using conventional medications, i.e., steroidal and non-steroidal anti-

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

inflammatory drugs, which are applied particularly to reduce the pain. However, the use of anti-inflammatory drugs has various side effects such as increasing the risk of stroke, peptic ulcer disease, and kidney failure if consumed in the long term (Wongrakpanich *et al*., 2018). This encourages the scientists to find the alternative of anti-inflammatory drugs that are effective and safe, especially for treating chronic inflammation (Cruz *et al*., 2016). Thus, in the last recent years, a number of clinical trials have demonstrated the effect of cocoa on inflammation. Polyphenols-containing cocoa play significant role in modulating the synthesis of pro- and anti-inflammatory metabolites (Ellinger and Stehle, 2016). Due to the recent growing body of literature discussing the effects of cocoa and chocolate as anti-inflammatory agent, a systematic review on the potential anti-inflammatory activity of cocoa and its derived products is required. The aims of this review were to examine the functionality of cocoa and chocolate as anti-inflammatory agent in clinical studies as well as to find the research gap in this research area.

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2. Materials and methods

2.1 Data sources, search strategy and selection process

This study was carried out by following the PRISMA Statement 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). A reputable electronic database namely Scopus were consulted. Keywords that were included and combined using "AND" were: "Cocoa", "Chocolate", "Polyphenol", "Anti-inflammatory". "Inflammation", "Anti-inflammatory", "Inflammatory". The framework of PICOS was used in this study in which there 5 components consisting of P (population/problem), I (Intervention/Indicator), C (Comparison), O (Outcome) and S (study design). The details of PICOS framework in addition to years and language used in this study are shown in Table 1. Meanwhile the selection process is shown Figure 1. As shown in Figure 1, after the selection process, there were 25 selected articles from 2061 articles firstly identified in the database.

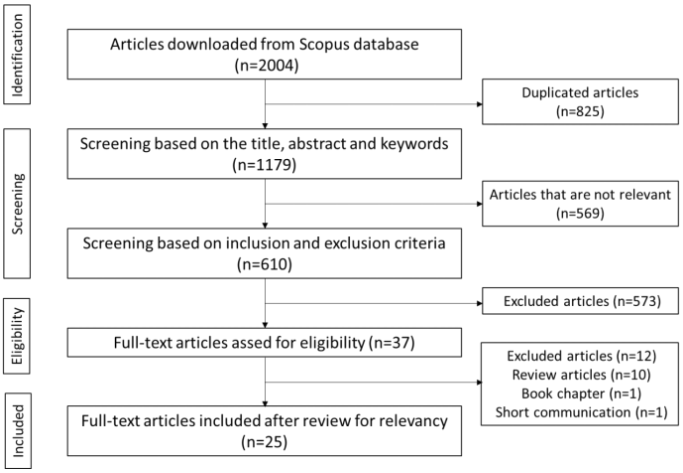

Figure 1. Selection process.

2.2 Data extraction, synthesis and report

In this study, the selected articles were tabulated into a table in which the tabulated data were then analyzed and synthesized. Synthesis of data included compiling, combining and making summaries of articles. The samples were grouped according to their health condition background, such as subjects without health issue (healthy subject), subjects with some health problems record and subjects with obesity. The data extracted from the articles included the condition of participants of the clinical trials, study design, intervention, duration and results which focused on the biomarkers of inflammation. The results were then reported by descriptive narrative to examine the functionality of cocoa and chocolate as anti-inflammatory agent in clinical studies as well as to find the research gap in this research area of study.

3. Results

The selection of articles resulted in 25 eligible articles for further analysis. The articles were then grouped in to three categories based on the condition of the subject, namely healthy subject, Subject with some health problems records and Subject with obesity (Tables 2, 3 and 4).

Table 1. Inclusion and exclusion criteria used in this study.

Criteria	Inclusion	Exclusion
Population	Articles from the Scopus database related to inflammation incidence in humans	Articles from the Scopus database which are related to inflammation incidence in humans
Intervention	There is an intervention by bioactive compounds from cocoa and chocolate.	There is no intervention by bioactive compounds from cocoa and chocolate.
Comparison	There is a control treatment	There is no control treatment
Outcome	There is anti-inflammatory effect from bioactive compounds from cocoa and its derived products.	There is no anti-inflammatory effect from bioactive compounds from cocoa and its derived products.
Study Design	Clinical study	Not clinical study
Years	From 2012 to 2021.	Before 2012
Language	English	Not English

https://doi.org/10.26656/fr.2017.8(S2).60

Table 2. Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in healthy subject.

Reference	Participants	Study Design	Intervention	Duration	Results
Gonzalez-Garrido *et al*. (2017)	N: 15 Condition: professional soccer player, has regular physical activity, has no chronic disease, not smoking, has no heart or respiratory disease, does not consume antioxidant supplements Age: 15-19 years BMI: 22.75±0.97 kg/m^2 Gender: Men	Case-controlled study	P: 25, 1 g cocoa powder "Hershey's" (FV = 1050 mg; EC = (×))	1 week (7 days)	**CRP** P = ~ .
Kuebler *et al*. (2016)	N: 68 (31 dark chocolate, 34 control) Condition: healthy, does not consume any drugs, not smoking, does not or rarely consume dark chocolate and food containing flavanol Age: 20-50 years BMI: 19.7-39.8kg/m^2 (dark chocolate), 18.7-36 kg/m^2 (control) Gender: Men	Placebo-controlled single-blind between-subjects design	C: 50 g chocolate placebo free flavanol (EC = 0 mg) P1: 50 g dark chocolate high flavanol 72% cocoa (EC = 125 mg)	120 mins	**IL -1β** C: ~ (0.06 → 0.06) P1: ↑ (0.05 → 0.06) Increase of P1 > C **IL-6** C: ↑ (0.23 → 0.89) P1: ↑ (0.30 → 0.70) Increase of P1 < C **IL-10** C: ↑ (0.16 → 0.28) P1: ↑ (0.19 → 0.22) Increase of C > P1
Vázquez-Agell *et al*. (2013)	N: 18 Condition: Healthy, has no diabetes mellitus, hypertension, heart disease, HIV, malnutrition, alcohol-induced liver disease and acute infection, not smoking Age: 19-49 years BMI: n.d Gender: 9 Men and 9 Women	Open, prospective, randomized, crossover	C: 250 mL milk P1: 40 g cocoa powder dissolved in milk (EC = 28.2 mg; CA = 8.4 mg)	6 hrs	**sE-selectin** C: ↑ P1: ↓ Decrease of P1 > C
Calderon-Garciduenas *et al*. (2013)	N: 18 Condition: not smoking and live in area exposed to tobacco, has no history of diseases including hearing and vision Age: 10.55 years (mean) BMI: 17.8 (mean) Gender: n.d	Pilot Study	P1: Warm milk with 30g cacao and *ad-libitum* sugar addition (FV = 680 mg)	15 days	**IL-1α** P_1: ↓ (6.5 → 6.2) **IL-1β** P_1: ↓ (3 → 2.9) **IL-2** P_1: ↑ (4.6 → 4.7) **IL-6** P_1: ↑ (2.5 → 3) **IL-10** P_1: ↑ (2.5 → 3) **TNF-α** P_1: ↑ (2.45 → 2.7)
Stote *et al*. (2012)	N: 20 Condition: not smoking, not pregnant and breastfeed, has no heart disease, diabetes, kidney, liver and cancer Age: 25-55 years BMI :> 27 kg/m^2 Gender: Men and Women	Randomized crossover design	C: 28 g cocoa powder (FV = 30 mg; EC = 4 mg; CA= 4 mg) P_1 (Low): 28 g cocoa powder (FV = 180 mg; EC = 34 mg; CA= 10 mg) P_2 (Medium): 28 g cocoa powder (FV = 400 mg; EC = 72 mg; CA= 24 mg) P_3 (High): 28g cocoa powder (FV = 900 mg; EC = 184 mg; CA= 52 mg)	5 days	**CRP** control (6.3 mg/ mL) P_1: ↓ (6.0 mg/mL) P_2: ↓ (5.6 mg/mL) P_3: ↓ (5.3 mg/mL) Decrease of P_3 > P_2 > P_1 **IL-6** Control (2.2 pg/ mL) P_1: ↑ (2.8 pg/mL) P_2: ↓ (2.1 pg/mL) P_3: ↑ (2.5 pg/mL)
Davison *et al*. (2012)	N: 14 Condition: healthy, not smoking, do not drink alcohol, caffeine, and not doing prolonged exercise Age: 22±1 years BMI: 71.6±1.6 kg/m^2 Gender: Men	Randomized, crossover-blind	C 71 g cacao free chocolate (EC = 0 mg) P1: 100 g dark chocolate "Nestle Noir" (EC = 96.8 mg; CA = 39.1 mg)	1 hr	**IL-6** C: ↑ (2.1 → 3.9) P1: ↑ (1.9 → 4.3) Increase of P1 > C

n.d.: Not determined

https://doi.org/10.26656/fr.2017.8(S2).60

Table 3. Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in subjects with some health problems record.

Reference	Participants	Study Design	Intervention	Duration	Results
Davis *et al*. (2020)	N: 18 Condition: has history of abdominal adiposity or type 2 diabetes for 5 years or more Age: ≥21 years BMI: >30.0 kg/m^2 Gender: 4 Men and 14 Women	Randomized crossover trial	C: 12 g cocoa placebo (negligible) P_1: 20 g cocoa powder (TP = 960 mg; EC = 40 mg)	hrs	**IL-6** C: ↓ (6.3 → 5,0) P1: ↑ (3.2 → 4.5) Decrease of P_1 < C **IL-1β** C: ↑ (3.5→ 4.2) P1: ↑ (3.0 → 4.0) Increase of P_1 > C **IL-18** C: ↑ (303→ 339) P1: ↓ (300 → 250) Decrease of P_1 > C
Munguia *et al*. (2019)	N: 60 (initial study) 74 (follow-up study) Condition: has history of sarcopenia, that not consume antioxidant supplement, cocoa powder, protein supplement and benzodiazepines. Age: 55-70 years (initial), 65- 90 years (follow-up) SMI: 8.87 kg/m^2 (L) and 6.42 kg/m^2 (P) Gender: Men and Women	Two-phase, randomized, double-blind	C: 22 g placebo powder P_1 (NF): 22 g Alkalized Cocoa powder without flavonoids (EC = 0 mg) P_2 (F): 22 g High flavonoid cacao powder (FV =179 mg; EC = 25 mg)	weeks	**TNF-α** If compare to control, P_1: ↓; P_2: ↓ Decrease of P2 > P1 **IL-6** If compare to control P_1: ↓; P_2: ↓ Decrease of P2 > P1
Jafarirad *et al*. (2018)	N: 44 Condition: has 2 types of diabetes Age: 30-60 Years BMI: 18.5-35 kg/m^2 Gender: Men and Women	Randomized parallel clinical trial	C: sample hadn't given P1: 30g dark chocolate (84%) from Kiam Chocolate Company (EC = n.d)	8 weeks	**hs-CRP** C: ↑ (3.2 → 4.21) P1: ↓ (4.2 → 3.9) Decrease of P1 > C **TNF-α** C: ↑ (24.6 → 25.65) P1: ↓ (34.33 → 23.42 Decrease of P1 > C **IL-6** C: ↑ (4.08 → 10.3) P1: ↓ (6.61 → 4.04) Decrease of P1 > C
Petrilli *et al*. (2016)	N: 92 Condition: HIV infected Age:19-59 Years BMI: 23.4±3.9 kg/m^2 Gender: Men and Women	Randomized, doble-blind, placebo-controlled crossover clinical trial	C: 65 g chocolate white P1: 65 g chocolate bar contains 36 g cocoa (TP = 2864 mg; EC = n.d)	105 days	**hs-CRP** C: ↑; P1: ↓ Decrease of P1 > C
Rostami *et al*. (2015)	N: 60 (32 groups DCG; 28 groups WCG) Condition: has history of hypertension and diabetes type 2, but can't accept insulin treatment and other medication within the last 2 months Age: 35-70 Years BMI: 30.12±2 kg/m^2 (DCG); 29.42±4.58 kg/m^2 (WCG) Gender: Men	Randomized, placebo-controlled, double-blind study	C (WCG) = 25 g chocolate white P_1 (DCG) = 25 g chocolate dark 83% cocoa (FV = 450 mg)	8 weeks	**CRP** C: ↓ (18.59 →17.21) P_1: ↓ (26.71 → 18.82) Decrease of P_1 > C

n.d.: Not determined

https://doi.org/10.26656/fr.2017.8(S2).60

Table 3 (Cont.). Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in subjects with some health problems record.

Reference	Participants	Study Design	Intervention	Duration	Results
Sarria *et al*. (2015)	N: 44 [24 Normocholesterolemic; 20 Hypercholesterolemic] Condition: has normocholesterolemic (<2000 mg/L) and hypercholesterolemic (>2000-2400 mg/L) Age: 18-55 Years BMI: <30 kg/m^2 Gender: 24 Women and 20 Men	Two controlled study	C: Milk P1: 15 g high soluble cocoa polyphenols (TP = 34.04 GAC/g; EC = 1.26 mg; CA = 0.47 mg)	4 weeks	**IL-1β** • Normo-C C: ↓; P1: ↓ Decrease of C = P1 • Hyper-C C: ~; P1: ↓ Decrease of P1> C IL-10 • Normo-C C: ↓; P1: ↓ Decrease of P1 > C • Hyper-C C: ↑; P1: ↓ Decrease of P1 > C
Basu *et al*. (2015)	N: 18 Condition: has history of diabetes type 2 ≥ 5 Years, without insulin treatment and obesity Age: 53-59 Years BMI: 35.3±2 kg/m^2 Gender: 4 Men and 14 Women	Randomized, double-blind, Crossover trial	C: 12 g placebo drinks replace with isolate protein milk (TP = 110 mg; EC = 0 mg) P_1: 20 g cocoa drinks (TP = 960 mg; FV = 480 mg; EC = 40 mg; CA = 18 mg)	6 hrs (Checked every 0; 30 60, 120 240, 360 min)	**CRP (C-Reactive Protein)** C: ~ P_1: ~ C = P_1
Horn *et al*. (2014)	N: 16 Condition: Patients with history of CAD (coronary artery disease) Age: 64 Years BMI: 28.8 kg/m^2 Gender: 13 Men and 3 Women	Randomized, Double-masked, cross-over	C (LoFl): 6 g low flavanol cocoa powder drinks from Mars Incorporated (FV = 9 mg; EC = 1 mg; CA = 2 mg) P_1 (HiFl): 6 g high flavanol cocoa powder drinks from Mars Incorporated (FV = 375 mg; EC = 59 mg; CA = 6 mg)	1 weeks	**EMP CD144$^+$ (endothelial microparticles)** C: ↓ (703 → 687) P1: ↓ (707 → 528) Decrease of P1 > C **EMP CD31$^+$/41$^-$** C: ↑ (1332 → 1467) P1: ↓ (1509 → 1086) Decrease of P1 > C **EMP CD41$^+$ (Platelet -derived Microparticles)** C: ↑ (4857 → 5418) P1: ↓ (5571 → 5236) Decrease of P1 > C
Parsaeyan *et al*. (2014)	N: 100 [50 persons control (c), 50 persons with treatment 1 (P1)] Condition: Patients with history of diabetes type 2, with cholesterol > 240 mg/dl, triglycerides > 200 mg/dl Age: 40-70 Years BMI: 28±0.5 kg/m^2 Gender: 50 Men and 50 Women	Randomized clinical control, parallel group	C: 10 g milk powder that dissolve in 250 mL boiled water (EC = n.d) P_1: 10 g cocoa powder with 10g milk powder that that dissolve in 250 mL boiled water (EC = n.d)	6 weeks	**CRP** If compare to control (C = 0.43) P_1 = ↓ (0.23) **IL-6** If compare to control (C = 2.5) P_1 = ↓ (1,7) **TNF-α** If compare to control (C = 2.64) P_1 = ↓ (1.38 mg/dl)

n.d.: Not determined

https://doi.org/10.26656/fr.2017.8(S2).60

Table 3 (Cont.). Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in subjects with some health problems record.

Reference	Participants	Study Design	Intervention	Duration	Results
Sarria *et al*. (2014)	N: 44 [24 normocholesteroleamic and 20 moderate hypercholesterolemic] Condition: has normocholesterolemic and mild hypercholesterolemic > 2000 mg/L Age: 18-55 Years BMI: 22.2 kg/m^2 (Women) and 25,15 kg/m^2 (Men) Gender: 24 Women and 20 Men	Randomized, controlled, cross-over, free-living study	C: 400 mL/days semi-skimmed milk (EC = 0 mg) P_1: 400 mL/ days semi-skimmed milk with cocoa from Nutrexpa (EC = 9.3 mg)	4 weeks	**IL-1β** • Normocholesterolemic C: ↓ (3.43 →2.62 pg/mL) P_1: ↓ (3.43 →2.47 pg/mL) • Hypercholesterolemic C: ↓ (2.80 →2.47 pg/mL) P_1: ↓ (2.80 →1.85 pg/mL) Decrease of P_1 > C (N/H) **IL-6** • Normocholesterolemic C: ↓ (4.30 →3.95 pg/mL) P_1: ↓ (4.30 →3.71 pg/mL) • **Hypercholesterolemic** C: ↓ (4.16 →3.89 pg/mL) P_1: ↓ (4.16 →3.58 pg/mL) Decrease of P_1 > C (N/H) **IL-8** • Normocholesterolemic C: ↓ (3.53 →2.80 pg/mL) P_1: ↓ (3.53 →2.80 pg/mL) • Hypercholesterolemic C: ↑ (3.08→3.41 pg/mL) P_1: ↓ (3.08→2.67 pg/mL) Decrease of C = P_1 (N) Decrease of P_1 > C (H) **IL-10** • Normocholesterolemic C: ↓ (13.55 →9.55 pg/mL) P_1: ↓ (13.55 →6.68 pg/mL) • Hypercholesterolemic C: ↓ (14.47→11.00 pg/mL) P_1: ↓ (14.47→7.88 pg/mL) Decrease of P_1 > C (N/H) **TNF-α** • Normocholesterolemic C: ↓ (6.24 →4.91 pg/mL) P_1: ↓ (6.24 →4.65 pg/mL) • Hypercholesterolemic C: ↑ (6.00→7.63pg/mL) P_1: ↓ (6.00→5.78 pg/mL)

n.d.: Not determined

https://doi.org/10.26656/fr.2017.8(S2).60

Table 3 (Cont.). Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in subjects with some health problems record.

Reference	Participants	Study Design	Intervention	Duration	Results
Martinez-Lopez *et al*. (2014)	N: 44 [24 normocholesterolemic and 20 moderate hypercholesterolemic] Condition: has normocholesterolemic (<200 mg/dl) and mild hypercholesterolemic (200-240 mg/l) Age: 18-55 Years BMI: 22.0-26.2 kg/m^2 Gender: 20 Men and 24 Women	Non-randomized, controlled, crossover	C: 400 mL/days semi-skimmed milk with (EC = 0 mg) P_1: 400 mL/days semi-skimmed milk with cocoa from Nutrexpa (EC = 9.3 mg)	4 weeks	**IL-1β** • Normocholesterolemic C: ↓ (3.40 →2.65 pg/mL) P_1: ↓ (3.40 →2.65 pg/mL) • Hypercholesterolemic C: ↓ (2.67 →2.65 pg/mL) P_1: ↓ (2.67 →2.11 pg/mL) Decrease of P_1 = C (N) Decrease of P_1 > C (H) **IL-6** • Normocholesterolemic C: ↓ (4.32 →3.82 pg/mL) P_1: ↓ (4.32 →3.73 pg/mL) • Hypercholesterolemic C: ↓ (3.94 →3.72 pg/mL) P_1: ↑ (3.94 →4.28 pg/mL) Decrease of P_1 > C (N) Decrease of C > P_1 (H) **IL-8** • Normocholesterolemic C: ↓ (3.61 →2.80 pg/mL) P_1: ↓ (3.61 →3.00 pg/mL) • Hypercholesterolemic C: ↓ (3.71→3.43 pg/mL) P_1: ↑ (3.71→4.57 pg/mL) Decrease of C > P_1 (N, H) **IL-10** • Normocholesterolemic C: ↓ (13.86 →9.90 pg/mL) P_1: ↓ (13.86 →8.90 pg/mL) • Hypercholesterolemic C: ↑ (11.73→12.40 pg/mL) P_1: ↓ (11.73→9.93 pg/mL) Decrease of P_1 > C (N, H) **TNF-α** • Normocholesterolemic C: ↓ (6.32 →4.91 pg/mL) P_1: ↓ (6.32 →5.91 pg/mL) • Hypercholesterolemic C: ↑ (5.95→7.63 pg/mL) P_1: ↑ (5.95→6.26 pg/mL) Decrease of C > P_1 (N) Increase of C > P_1 (H)
Flammer *et al*. (2012)	N: 20 (10 Control and 10 Treatment 1) Condition: has history of congestive heart failure Age: 59±3 Years BMI: <30 kg/m^2 Gender: 17 Men and 3 Women	Randomized, double-blind, parallel group	C: 28.4 g cocoa free chocolate liquor from "Nestle" (EC = 0mg) P1: 40 g high flavanol dark chocolate from "Nestle Noir Intense" (EC = 0.9 mg/g)	2 hrs	**CRP** C: ~ (4.4 → 4.4) P1: ↑ (1.4 → 1.5) Increase of P1 > C
Nogueira *et al*. (2012)	N: 20 Condition: Diagnozed with level 1 hypertension Age: 18-60 Years BMI: 25-34.9 kg/m^2 Gender: 10 Men and 10 Women	Pre-post trial	P1: 50 g chocolate with 70% cocoa (TP = 2135 mg; EC = n.d))	4 weeks	Hs-CRP: P1: ↓ (0.93 → 0.61) TNF-αP1: ↑ (17.51 → 18.96) IL-6: P1: ↓ (87.87 → 69.40)

n.d.: Not determined

https://doi.org/10.26656/fr.2017.8(S2).60

Table 4. Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in subjects with obesity.

Reference	Participants	Study Design	Intervention	Duration	Results
Eskandari *et al*. (2020)	N: 48 Condition: Obesity, no weight loss > 2 kg within last 6 months, do physical activities < 1 hrs per weeks within last 1 years Age: 13-17 Years BMI: 31.3-33.2 kg/m^2 Gender: 307 Men and 367 Women	Randomized, group control	C: 30 g white chocolate (placebo) with 0% cocoa (TP = 0 mg; EC = 0 mg) P1: 30 g dark chocolate with 83% cocoa (TP =2650 mg; EC = 160 mg	6 weeks	**hs-CRP** C: ↑; P1: ↓ Decrease of P1 > C **TNF-α** C: ↑; P1: ↓ Decrease of P1 > C **IL-6** C: ↓; P1: ↓ Decrease of P1 > C
Vieira *et al*. (2016)	N: 60 Condition: Obesity, not taking anti-inflammatory medicines, taking digestive medications or antibiotics within the last 6 months Age: 20-50 Years BMI: 25-35 kg/m^2 Gender: Women	Double-blind, randomized clinical trial	C: 54.1 g cocoa with UBF (Unripe Banana Flour) (EC = n.d) P_1: 39.1 g drinks with cocoa (EC = n.d)	6 weeks	**IL-17** C: ↓ (41.81→ 27.16 pg/mL) P_1: ↓ (49.17 →22.15 pg/mL) Decrease of P_1 > C **TNF-α** C: ↓ (3.07 → 2.61 pg/mL) P_1: ↑ (0.57 → 1.21 pg/mL) Decrease of C > P_1 **IL-10** C: ↓ (0.82 → 0.52 pg/mL) P_1: ↑ (0.57 → 0.61 pg/mL) Decrease of P_1< C **IL-6** C: ↑ (2.13 → 2.83 pg/mL) P_1: ↑ (2.00 → 3.08 pg/mL) Increase of P_1 > C **IL-4** C: ↑ (0.59 → 0.74 pg/mL) P_1: ↑ (0.42 → 0.78 pg/mL) Increase of P_1 > C **IL-2** C: ↑ (1.25 → 3.16 pg/mL) P_1: ↑ (0.75 → 2.29 pg/mL) Increase of P_1 > C
McFarlin *et al*. (2015)	N: 24 (normal = 10; overweight = 7, obese = 7) Condition: divided into 3 groups; normal, overweight, obese. Age: 19-35 Years BMI: normal =20-24.9; overweight = 25-29.9; obese =30-30.0 kg/m^2 Gender: Women	Double-blind, crossover design	C: Plasebo bar P_1 = natural cocoa bar contains 12.7 g cocoa (EC = 48 mg; CA = 13.6 mg)	4 weeks	**EMP** C: ↓ (normal and overweight) ~ (Obese) P_1: ↓ (Overweight and Obese) ↑ (Normal) Decrease of P_1 > C **E-selectin** C: ↓ (Overweight) ↑ (normal and obese) P_1: ↓ Decrease of P_1 > C

n.d.: Not determined

https://doi.org/10.26656/fr.2017.8(S2).60

Table 4 (Cont.). Anti-inflammatory effect of cocoa (*Theobroma cacao* L.) in subjects with obesity.

Reference	Participants	Study Design	Intervention	Duration	Results
West *et al*. (2014)	N: 30 Condition: Overweight or Obesity Age: 40-64 Years BMI: 25-37 kg/m^2 Gender: Men and Women	Randomized, placebo-controlled, cross-over study	$C_{chocolate}$: low-flavanol chocolate bar (FV = 1 mg; EC = 0.9 mg; CA = 0.2 mg) C_{cocoa}: No sugar cocoa-free drinks (FV = 2 mg; EC = 0 mg; CA = 0 mg) $P_{chocolate}$: Dark Chocolate from "Hersheys extra dark chocolate" 37 g/days (FV = 347 mg; EC = 32.6 mg; CA = 5.9 mg) P_{cocoa}: No sugar cocoa drinks with total of 22 g/days (FV = 440 mg; EC = 41 mg; CA = 18.7 mg)	4 weeks (2 weeks chocolate and 2 weeks cocoa)	**IL-1** C: ↑ (0.09 → 0.16) P_1: ↑ (0.09 → 0.11) Increase of C > P_1 **IL-6** C: ↓ (1.24 → 0.99) P_1: ↓ (1.24 → 1.18) Decrease of C > P_1 **TNF-α** C: ↑ (0.99 → 1.12) P_1: ↑ (0.99 → 1.03) Increase of C > P_1
Esser *et al*. (2014)	N: 41 Condition: Obesity, not smoking, normoglycemic, and without any serious medical condition or hypertension diagnozed Age: 45-70 Years BMI: 25-32 kg/m^2 Gender: Men	Double-blind randomized crossover	C: 70 g low flavanol chocolate with 58% cocoa (FV = 259 mg; EC = 97 mg) P1: 70 g chocolate with 58% cocoa (FV = 1078 mg; EC = 349 mg)	4 weeks	**E-selectin**: C: ↑; P1: ↑ Increase of P1 > C **IL-1β**: C: ↓; P1: ↓ Decrease of P1 > C **IL-6**: C: ↓; P1: ~ Decrease of C > P1 **IL-8:** C: ↑; P1: ~ Increase of C > P1 **TNF-α**: C: ↑; P1: ↓ Decrease of P1 > C **CRP**: C: ↓; P1: ↑ Decrease of C > P1
Renzo *et al*. (2013)	N: 20 Condition: has NWO (Normal Weight Obese) syndrome Age: 20-40 Years BMI: < 25 kg/m^2 Gender: Women	Case-control study	P1: 100 g dark chocolate with 70% cocoa (EC = 908 mg; CA = 444 mg)	7 days	**hs-CRP**: P1: ↑ (0.97 → 1.51) **IL-1α**: P1: ↓ (2.74 → 2.70) **IL-1β**: P1: ↓ (1.17 → 0.83) **IL-6**: P1: ↓ (1.81 → 1.22) **TNF-α**: P1: ↓ (0.16 → 0.13)

n.d.: Not determined

3.1 Healthy subject

Table 2 shows the effect of cocoa and its derived products on inflammation in subjects with healthy conditions in which the subjects do not have congenital diseases, do not take drugs are not breastfeeding and are not pregnant. In this study, blood samples were analyzed, namely plasma. In the study of Gonzalez-Garrido *et al*. (2017), the biomarker of C-reactive protein (CRP) was used as the indicator. Samples used in this study were cocoa powder with flavonoid content of 1050 mg. After 7 days treatment, there were no significant changes that occurred in the inflammatory biomarker of CRP.

In the study of Kuebler *et al*. (2016), interleukin (IL) was used as the biomarkers in which the level of IL-1β, IL-6, IL-10 of blood samples were analysed. In this study, 50 g of flavanol-free placebo chocolate (EC = 0 mg) as control and 50 g of high flavanol 72% cocoa (EC = 125 mg) as treatment were used by applying the research design of placebo-controlled single-blind between subjects. The results showed that by treatment of chocolate increase of IL-1β, IL-6 and IL-10 were shown. However, it is important to note that, there was no significant change in the level of IL-1β of control. Also, by the treatment, the increase of IL-6 and IL-10 was smaller than the control. Vázquez-Agell *et al*. (2013) used sE-selectin as the biomarker. Cocoa powder (40 g) containing 28.2 mg of epicatechin and 8.4 mg of

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catechin was mixed with milk. After the treatment, the subjects showed a decrease in the level of sE-selectin. Meanwhile, the subject administered with control (250 ml of milk without cocoa content) showed an increase in the level of sE-selectin.

In accordance with other studies, Calderon-Garciduenas (2013) used IL-1α, IL-1β, IL-2, IL-6, IL-10 and TNF-α as the indicators. In this research, it was shown that milk added with 40 g of cocoa powder significantly reduced the level of IL-1α dan IL-1β and at the same time increased IL-2, IL-6, IL-10 dan TNF-α. In another study, Stote *et al*. (2012) used CRP and IL-6 as the inflammatory biomarker. In this research, cocoa powder with different level of flavanols were used in which control, low, medium and high levels of flavanols were equal to 30, 180, 400 and 900 mg flavanols. The decrease of CRP was in line with level of flavanols administered in the subject. Davison *et al*. (2012) used IL-6 as the inflammatory biomarker The subjects administered with 71 g of chocolate without no epicatechin content and 100 g dark chocolate containing epicatechin of 96.8 mg and catechin of 39.1 mg. By using randomized, crossover-blind study, it was found that the treatment using chocolate containing epicatechin resulted in higher level of IL-6 than control.

3.2 Subject with some health problems records

In Table 3, there are 13 studies examining subjects with some health problems records such as coronary artery disease (CAD), diabetes, hypertension and cholesterol. Blood plasma was sampled for measuring the biomarkers. Davis *et al*. (2020) used the randomized crossover trial to examine the anti-inflammatory activity of 20 g cocoa powder containing polyphenols of 960 mg and epicatechin of 40 mg. IL-6, IL-1β and IL-18 were used as the biomarkers. Administered with the samples, all the biomarkers increased significantly. Munguia *et al*. (2019) used 22 g of placebo powder as control, 22 g of non-alkalised cocoa powder without epicatechin content and 22 g of cocoa powder containing 179 mg and flavonoid and 25 mg of epicatechin. The samples were subjected to the people with sarcopenia for 12 weeks using two-phase, randomized, double-blind research design. It was shown that the levels of TNF-α and IL-6 decreased significantly after the treatment using cocoa powder containing flavonoids.

Jafarirad *et al*. (2018) investigated anti-inflammatory effect of 30 g dark chocolate administered for 8 weeks to the subject with type 2 diabetes. The research design of randomized parallel clinical trial was used and the results showed that CRP, TNF-α and IL-6 biomarkers significantly decreased. Petrilli *et al*. (2016) worked with patient with HIV. After treatment with 65 g of dark chocolate, CRP significantly increase while treatment using 65 g of white chocolate resulted in a decrease of CRP. Rostami *et al*. (2015) worked with subjects with hypertension and type 2 diabetes. Treatment using 65 g of dark chocolate containing 450 mg flavanols resulted in less significant decrease on CRP than treatment using 65 g of white chocolate. On the contrary, Basu *et al*. (2015) worked with patients with type 2 diabetes for more than 5 years found that CRP remained stable after the treatment of 20 g cocoa drink containing 960 mg of polyphenols, 40 mg of epicatechin and 18 mg of catechin for 6 hours. Parsaeyan *et al*. (2014) showed that in patients with type 2 diabetes 10 g of cocoa powder diluted in 250 ml of water significantly reduced CRP, IL-6 and TNF-α.

3.3 Subject with obesity

In Table 4, there are 6 studies examining the blood plasma of subjects with obesity after treatment with cocoa or cocoa derived products. Eskandari *et al*. (2020) reported that 30 g of dark chocolate containing 160 mg of epicatechin significantly reduced CRP, TNF-α dan IL-6 after 6 weeks treatment McFarlin *et al*. (2015) showed that 12.7 g cocoa samples containing 48 mg of epicatechin and 13.6 catechin showed positive anti-inflammatory responses as indicated by EMP and E-selectin biomarkers. West *et al*. (2014) revealed by using randomised, double-blind placebo-controlled, cross-over study that 37 mg of chocolate bars containing 347 mg of flavanols, 32.6 mg epicatechin and 5.9 mg resulted anti-inflammatory effect as indicated by IL-1, IL-6 and TNF-α biomarkers. A similar result was found by treatment of cocoa drink 22 g/day for 4 weeks in which the drink contained 440 mg of flavanols, 41 mg of epicatechin and 18.7 mg of catechin. Anti-inflammatory effect of cocoa was also shown by Esser *et al*. (2014) by using biomarkers of E-selectin, IL-1β, IL-6, IL-8, TNF-α and CRP. Meanwhile, Renzo et al. (2013) demonstrated that 100 g of dark chocolate containing 908 mg of epicatechin and 444 mg of catechin administered to the subject for 7 days resulted in a higher CRP and lower IL-1α, IL-1β, IL-6 and TNF-α.

4. Discussion

Many studies have shown that after consuming cocoa or cocoa-derived products could reduce inflammation. The conclusion based on the observation on the level of inflammatory biomarkers. To determine inflammation, pro- and anti-inflammation biomarkers can be used in which the most widely used pro-inflammatory biomarkers are IL-1α, IL-1β, IL-2, IL-6, IL -8, IL-17, IL-18 TNF-α, CRP, EMP CD144+, EMP CD31+/41-, EMP CD41+ dan sE-selectin while the most widely used anti-inflammatory biomarkers are IL-4 dan

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IL-10.

As well-explained by Dinarello (2000), pro-inflammatory biomarkers typically can cause a disease or pathological condition to get worse. Meanwhile, anti-inflammatory biomarkers typically can block cytokines that are harmful to the body so that they can relieve infection, reduce inflammation and can help the healing process. Hence, the higher the number of pro-inflammatory biomarkers produced, the worse the inflammation. On the contrary, the higher the number of the anti-inflammatory biomarkers obtained, the faster the healing process.

Anti-inflammatory biomarkers such as IL-4 and IL-1 significantly increased after being treated with cocoa or its derived products (Calderon- Garciduenas *et al*., 2013; Vieira *et al*., 2016). However, a contrast results were found in which there is no significant change in the level of anti-inflammatory biomarker after treatment (Sarria *et al*., 2014; Martinez-lopez *et al*., 2014; Sarria *et al*., 2015; Kuebler *et al*., 2016). Nevertheless, anti-inflammatory activity can also be determined by measuring the reduction of pro-inflammatory cytokine. As demonstrated in some journals, treatment of cocoa and its derived product significantly reduced pro-inflammatory biomarkers including IL6, IL1α, CRP, IL18, IL1β, IL6, IL17, IL1 and TNFα. However, some results also showed that pro-inflammatory biomarkers did not significantly change after treatment (Table 2, 3 and 4). One of the reasons behind this result is that testing on humans (clinical study) is more difficult than checking on laboratories and animals (animal study). In laboratory and animal checks, it is necessary to consider that experimental models can be made under highly controlled and uniform conditions such as environmental conditions, food, age and so on. Whereas in clinical studies, research subjects are difficult to fully control (Goya *et al*., 2016).

In-depth investigation as per category of subject found that in the 6 articles with healthy subjects, there were 14 biomarkers used in the studies. From a total of 14 biomarkers, it is known that 6 biomarkers showed positive results on the effect of cocoa or chocolate derivative products on inflammation (42.86%). In the category of subject with some health problems records, 22 of 33 biomarkers indicated that cocoa or chocolate derivative products have a positive effect on inflammation (66.67%). Meanwhile, in subjects with obesity, it is known that 16 out of 25 biomarkers indicated that cocoa and chocolate derivative products have a positive influence on inflammation (64%). Based on the above results, from a total of 25 articles, 44 of 72 biomarkers (about 61%) indicated that cocoa and chocolate derivative products had an anti-inflammatory effect.

It is a well-known fact that cocoa contains a very high number of polyphenolic compounds, namely flavonoids, especially flavanols (flavan-3-ols). The main flavanols in cocoa are epicatechin and catechin which are classified as flavanol monomers and procyanidins as flavanol oligomers (Katz *et al*., 2011). Flavanols can effectively modulate the inflammatory process by affecting endothelium-derived NO synthesis and metabolism, cytokine production and eicosanoid metabolism through specific actions on peripheral blood mononuclear cells (PBMCs). Also, flavanols can reduce the activation of NF-kB which is one of the major induced transcription factors to control the inflammatory response, cell proliferation/growth, and cellular adhesion by regulating the transcription of several pro-inflammatory cytokines, prostaglandins and NO (Selmi *et al*., 2008). In fact, the anti-inflammatory effect of cocoa is influenced by the dose of ingested cocoa flavanols. Unfortunately, some studies did not include the dose of flavanol used. The flavanol content in cocoa is also strongly correlated with the amount of catechins and epicatechins which are responsible for the anti-inflammatory effect (Ellinger and Stehle, 2016). This must be taken into account in the future studies.

Another attention that must be considered in the future research is that in the clinical study stage, the results may have inconsistencies as compared to the pre-clinical studies. This is due to the influence of the human body's response to inflammation. Every human being has a different body response that is influenced by genetic polymorphisms and epigenomic events. Genetic polymorphisms can alter nutrigenetic effects, namely responses to food components by influencing absorption and metabolism (Milner 2006). In depth investigation in this area, therefore, might be interesting in the future.

Moreover, methodological differences as well as subject grouping such as age, BMI, health status, stage of vascular inflammation, type of sample (food matrix), flavanol dose (epicatechin and catechin) and biomarkers may result in diverse results. Also, this condition makes difficulties in comparing between studies. Thus, this can be a research gap that must be fulfil by researchers. Another topic that may be interesting in the future is that investigating the intercorrelation between food matrix and anti-inflammatory activity. This is based on the fact that the type of sample or food matrix can influence the results of inflammatory biomarkers. As such, foods with a liquid matrix such as cocoa drinks may have a higher bioavailability than more complex structured foods such as chocolate bars, so that the flavanol content in cocoa drinks is easier for the body to absorb (Muhammad *et al*., 2019).

https://doi.org/10.26656/fr.2017.8(S2).60

5. Conclusion

To conclude, it has been evidenced that in clinical studies, bioactive compounds of cocoa particularly flavan-3-ol significantly reduce the inflammation which is shown by 61.11% of inflammatory biomarkers after getting intervention. This result was demonstrated in different group of samples namely healthy subject, subjects with some health problems record and subjects with obesity. The mode of action of the cocoa flavanols for reducing the inflammatory disorder is by modulating the activation of transcript regulating pro-inflammation cytokine (NF-kB), prostaglandin and NO. Nevertheless, there are still some research gaps to fulfil in order to complete the puzzle of information regarding the potential anti-inflammatory activity of cocoa. As such, this research can be continued by conducting meta-analysis to draw a more accurate conclusion. Also, there is no existing study grouping research subject based on BMI and age. These two factors may have significant effect on the effectiveness of cocoa flavanols as anti-inflammatory agent. Therefore, further research in this area is required.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This research was supported by Universitas Sebelas Maret (Contract No.: 228/UN27.22/PT.01.03/2023).

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