

## ANALYSIS OF INCREASED C-REACTIVE PROTEIN LEVELS IN BLOOD SERUM OF RATTUS NORVEGICUS WISTAR STRAIN DUE TO INTAKE OF POLYETHYLENE MICROPLASTICS

Alvin Julian<sup>1</sup>, Niluh Suwasanti<sup>2</sup>, Yudhiakuari Sincihu<sup>3</sup>, Bernadette Dian Novita<sup>4</sup>, Kevin Samsudin<sup>5</sup>

\*Corresponding author's email: [alvinjulian@ukwms.ac.id](mailto:alvinjulian@ukwms.ac.id)

DOI: <https://doi.org/10.33508/jwmj.v6i2.5511>

### ABSTRACT

**Introduction:** The widespread use of plastic and poor management of plastic waste is an environmental problem and has an impact on human health. The most commonly found microplastic contaminant is polyethylene polymer. Ingested microplastic particles will undergo an endocytosis mechanism and be absorbed into the bloodstream. This then triggers an increase in reactive oxygen species (ROS) which induces oxidative stress and results in an inflammatory response. This study tries to analyze the effect of administering microplastics on increasing blood serum C-reactive protein as a biomarker and marker of the inflammatory response due to microplastics.

**Method:** The experimental analytical research used 42 Rattus Norvegicus Wistar Strain animals, which were divided into 5 experimental groups and 1 control group. Quantitative data measurements/collection were carried out at two times (pre-post-test control group design) and analyzed by non-parametric comparison using the Friedman Test to see the increase in C-Reactive Protein (CRP) levels in the blood serum of Rattus norvegicus Wistar Strain before and after being given intake polyethylene microplastics.

**Result:** In all groups X0-X5, the results of the comparative test using the Friedman test showed a significant value of  $P = 0.000$  ( $P < 0.05$ ), so it can be concluded that there is a difference in pre and post experimental CRP levels.

**Discussion:** The increase in serum CRP levels from the control group to the X5 treatment group may be due to oxidative stress mechanisms, especially in hepatocyte cells, smooth muscle cells, macrophage cells, endothelial cells, lymphocyte cells and adipocyte cells which induce the production of CRP protein, especially native C-reactive protein (nCRP) and monomeric C-reactive protein (mCRP). The difference in serum CRP levels was significant ( $P = 0.000$ ), where there was an increase in serum CRP levels post treatment (post experimental) from the lowest mean of 0.05 mg/L (pre-experimental) to the highest of 0.98 mg/L on average, indicating that administration of microplastic intake at the dose studied had an impact on increasing serum CRP levels in response to an inflammatory reaction. The limitation of this research is that there are no journals that examine microplastics on serum CRP levels, resulting in a lack of research that can be used as a reference or comparative theory.

**Conclusion:** Administration of Microplastic Polyethylene at the dose studied in the treatment group caused a significant increase in Rattus Norvegicus Wistar Strain Serum CRP levels.

---

<sup>1</sup>) Department of Surgery and Anaesthesiology, Faculty of Medicine, Widya Mandala Surabaya Catholic University

<sup>2</sup>) Department of Clinical Pathology, Faculty of Medicine, Widya Mandala Surabaya Catholic University

<sup>3</sup>) Department of Public Health, Faculty of Medicine, Widya Mandala Surabaya Catholic University

<sup>4</sup>) Department of Pharmacology, Faculty of Medicine, Widya Mandala Surabaya Catholic University

<sup>5</sup>) Department of Physiology and Biochemistry, Faculty of Medicine, Widya Mandala Surabaya Catholic University

**Keyword:** microplastic, inflammatory response, c-reactive protein

## INTRODUCTION

We are currently entering the plastic era.<sup>1</sup> The widespread use of plastic and poor management of plastic waste has become an environmental problem and has an impact on human health.<sup>2</sup> Microplastics can easily be found in various food products such as drinking water (94.37 particles/L), honey (54 particles/L), sugar (0.44 particles/g), table salt (140.2 particles/kg), sardines (0.3 mg/g), and vegetables (52,050-233,000 particles/g).<sup>3,4,5,6,7</sup> It is estimated that adult consumption of microplastics reaches 126-142 particles per day unintentionally.<sup>7</sup> According to Senatehirajah et al (2020), humans swallow around 0.1-5 grams of microplastics/week.<sup>8</sup> The most common microplastic contaminants found is a polyethylene polymer.<sup>9</sup>

Ingested microplastic particles will enter the gastrointestinal tract and undergo an endocytosis mechanism by M cells in intestinal lymphoid tissue, namely Peyer's patches, so that they are absorbed into the bloodstream.<sup>10,11</sup> This then triggers an increase in reactive oxygen species (ROS) which causes oxidative stress. In acute and chronic conditions, oxidative stress will result in inflammation or injury to cells, tissues and organs, one of which is endothelial dysfunction.<sup>10</sup> Many cases of

inflammation are caused by high levels of reactive oxygen species (ROS), which are oxidants. C-Reactive Protein (CRP) is a protein belonging to the pentraxins family, which is a homopentameric acute phase inflammatory protein, known as native/pentameric CRP (nCRP/pCRP) which can irreversibly dissociate at sites of inflammation and infection into 5 separate monomers, known as with the term modified/monomeric CRP (mCRP). Apart from functioning as a biomarker, C-Reactive Protein (CRP) can be used to non-specifically monitor the presence of local or systemic disease. CRP levels will usually increase after trauma, an infection, especially due to bacteria, and inflammatory processes.<sup>12,13</sup> CRP levels can also reflect the level of inflammation that occurs, the concentration of CRP in the blood can experience a visible increase within 2 hours after the onset of symptoms and reaches peak values within 48 hours.<sup>14,15,23</sup>

Research on the toxicity of microplastics to biological cells has been the focus of our research since 2019, and has resulted in various Scopus indexed articles. Experimental research on microplastic toxicity always uses experimental animals because ethical

aspects cannot be carried out on humans. *Rattus norvegicus* is a substantial genetic homologue to humans so it can model the occurrence of disease in humans. Therefore, researchers tried to analyze the effect of administering microplastics on increasing blood serum C-reactive protein as a biomarker and marker of the inflammatory response due to microplastics.

## POLYETHYLENE MICROPLASTIC

Microplastics (MPs) are plastic particles measuring less than 5 mm which can be produced intentionally (primary microplastics) or produced from larger plastics (secondary microplastics), and enter the environment through various anthropogenic activities and natural pathways, thereby polluting the ecosystem and the entire food chain.<sup>17</sup> Molded MPs are formed from common types of plastics such as polyethylene, polypropylene, polystyrene, nylon, polyester, acrylic, polyoxymethylene, polyvinyl alcohol, polyvinyl chloride, poly methyl acrylate, polyethylene terephthalate, and polyurethane, etc. MPs can be of various types based on their source. Common forms are: i) Microbeads are small polyethylene beads commonly found as exfoliants in health products and cosmetics also categorized as microplastics, (ii) Scrubbers, namely MPs produced from industrial

cleaning agents, (iii) Nurdles, namely MPs formed from plastic raw materials, (iv) Plastic powder, which is produced from powder used in industrial printing, (v) Fiber, namely MP formed from clothing and cigarette filters, (vi) MPs produced from the foam used for packaging and also from Styrofoam cups, as well as worn tires (Figure 1).<sup>17</sup>

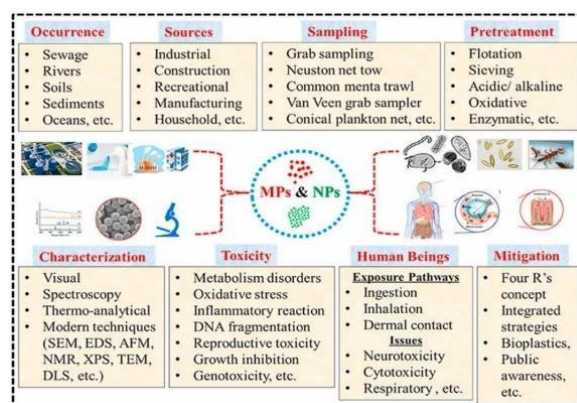


Figure 1. Mechanism of MPs inducing ROS.<sup>65</sup>

When large plastic particles are intentionally produced for use in products (e.g. cosmetics, such as exfoliants or toothpastes) or by industry (e.g. air blasting), they are called primary microplastics.<sup>18</sup> MPs formed from the degradation of bulk plastics are called secondary microplastics. The degradation of macroplastics into smaller forms, namely microplastics (MPs = < 5 mm) or Nanoplastics (NPs = < 1 μm) is a potential threat to terrestrial and marine ecosystems as well as human health because it has a direct impact on organs and activates a large number of intracellular signals, which can cause cell death. There is mounting evidence supporting the serious toxicity

caused by MP/NPs at all levels of biological complexity (biomolecules, organelles, cells, tissues, organs, and organ systems) and the involvement of reactive oxygen species (ROS) in these processes.<sup>19</sup>

Exposure to MPs can occur through oral consumption, inhalation (per inhalation), and skin contact due to the presence of microplastics in products, food ingredients, and air. Microplastics have been identified as being present in the atmospheric, aquatic and terrestrial environments, as well as drinking water and food products for human consumption, so they have the potential to cause adverse health impacts if ingested and/or inhaled. However, little is known about the impact of microplastics on human health.<sup>19</sup> The increasing consumption of plastics, coupled with their persistent nature, has led to increased human exposure to microplastics. In conditions of high concentration or high individual susceptibility, microplastics can cause inflammatory lesions, which originate from potential surface interactions with tissue. Exposure to microplastics can cause particle toxicity, accompanied by oxidative stress, the emergence of inflammatory lesions, and increased uptake and/or tissue translocation. The immune system's inability to eliminate synthetic particles can lead to chronic inflammation and increase the risk of neoplasia. Additionally,

microplastics can release their constituents, adsorbed contaminants, and other pathogenic organisms.<sup>20</sup>

The increasing incidence of neurodegenerative diseases, immune disorders, and cancer may also be related to increased exposure to environmental contaminants by microplastics. Nevertheless, knowledge about the toxicity of microplastics is still limited and is largely influenced by exposure concentration, particle properties, adsorbed contaminants, tissues involved, and individual susceptibility, thus requiring further research.

### **C-REACTIVE PROTEIN (CRP)**

C-Reactive Protein (CRP) is a homopentameric acute phase inflammatory protein, which is conserved in plasma. CRP was first discovered in 1930 by Tillet and Francis while investigating the serum of patients suffering from acute Pneumococcus infections and was named based on its reaction with the capsular (C)-polysaccharide of Pneumococcus.<sup>21</sup> CRP is produced as a homopentameric protein, called native CRP (nCRP) or natural CRP, which can irreversibly dissociate at sites of inflammation and infection into five separate monomers, called monomeric CRP (mCRP). CRP is synthesized primarily in liver hepatocytes but also by smooth muscle cells, macrophages,

endothelial cells, lymphocytes, and adipocytes. In the presence of calcium, CRP will bind to polysaccharides such as phosphocholine (PCh) in microorganisms and trigger the classical complement pathway of innate immunity by activating C1q.<sup>22</sup>

CRP has many homologues in vertebrates and several others in invertebrates<sup>23</sup> and is a member of the pentraxin family, which includes other structurally related molecules such as serum amyloid A.<sup>24</sup> The pentameric protein, termed native CRP (nCRP), is characterized by a discoidal configuration of five identical non-covalently bound protein subunits, each 206 amino acids long with a single molecular mass of approximately 23 kDa. These five subunits are located in the same orientation around the central pore and are arranged in a characteristic "lectin fold" with two layers of beta sheets.<sup>25</sup> Each subunit is located at the PCh binding site facing the "fit" side of the nCRP molecule.<sup>26</sup> The molecule has a ligand-binding surface that is characterized by having two calcium ions per protomer.

Calcium ions are important for ligand stability and binding. Meanwhile, the "opposite" side will interact with the C1q aspect of the complement pathway and interact with the Fc receptor.<sup>27</sup> CRP is first synthesized as a monomer and then

assembled into a pentamer in the endoplasmic reticulum of the source cell. Pentameric CRP can be permanently dissociated, with the resulting free subunit called monomeric or modified CRP (mCRP). Dissociation of nCRP into free subunits have been observed at high urea concentrations or high temperatures in the absence of calcium.<sup>28, 29</sup>

The average serum CRP level in healthy Caucasian individuals is approximately 0.8 mg/L, but this baseline number can vary greatly among individuals due to other factors, including polymorphisms in the CRP gene.<sup>30</sup> The human CRP gene can be found at 1q23.2 on the long arm of chromosome 1, and to date no allelic variations or genetic deficiencies have been found for this gene although several polymorphisms have been identified.<sup>31</sup> For example, up to 50% of the CRP base variance is associated with the number of dinucleotide repeats found in the region. intronic from normal genes.<sup>25</sup> Pankow et al. found evidence that variations in blood CRP levels between individuals of 35-40% are genetic/inherited factors.<sup>32,33</sup>

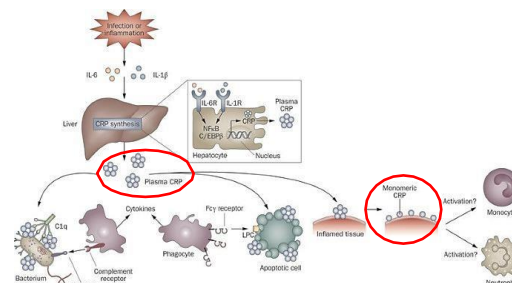
## **THE ROLE OF C-REACTIVE PROTEIN (CRP) IN THE INFLAMMATORY PROCESS**

C-Reactive Protein (CRP) is an acute inflammatory protein that will

increase up to 1,000 times in conditions of infection or inflammation. The mCRP molecule can be distinguished from nCRP based on its antigenic and biological differences. The biological properties that differ between mCRP and nCRP are that nCRP often shows more anti-inflammatory activity compared to mCRP. The nCRP isoform activates the classical complement pathway, induces phagocytosis, and promotes apoptosis. On the other hand, mCRP induces chemotaxis and recruitment of circulating leukocytes to areas of inflammation and can delay apoptosis (Figure 2).<sup>33</sup>

The nCRP and mCRP isoforms work in opposite directions, respectively inhibiting and inducing the production of Nitric Oxide (NO) through downregulation and upregulation of eNOS as well as the production of proinflammatory cytokines. The explanation above shows that in general nCRP tends to show more anti-inflammatory activity than the mCRP isoform, this is because nCRP limits the formation of the membrane attack complex (MAC) and complement C5a, thereby inhibiting alternative complement activation.<sup>34</sup> In contrast, mCRP can have marked pro-inflammatory properties both in vitro and in vivo. mCRP increases IL-8 and MCP-1 production by promoting monocyte chemotaxis and recruitment of circulating leukocytes to areas of

inflammation via Fcγ-RI and Fcγ-RIIIa signaling.<sup>34</sup>



**Figure 2. The role of native C-reactive protein (nCRP) and monomeric C-reactive protein (mCRP) in inflammation, infection, and pathological conditions.**<sup>33</sup>

Khreiss et al. provides clinical evidence that nCRP suppresses platelet attachment to neutrophils, whereas mCRP enhances this interaction. This difference in function can be explained by the two isoforms binding to different types of Fcγ receptors involved in the signaling process. The mCRP isoform uses immune complex binding with low-affinity immunoglobulin G (IgG) receptors called FcγRIIIb (CD16b) on neutrophils and FcγRIIIa (CD16a) on monocytes, while nCRP binds to the low-affinity IgG receptor FcγRIIa (CD32).<sup>33,35</sup> Ji et al. found that C-reactive protein or nCRP first dissociates into subunits while retaining some of the native conformation before completely dissociating into mCRP. This intermediate, called mCRPm, is formed when nCRP is bound to the cell membrane and then dissociates, allowing the subunits to maintain several conformations before dissociating completely into mCRP subunits upon detachment from the

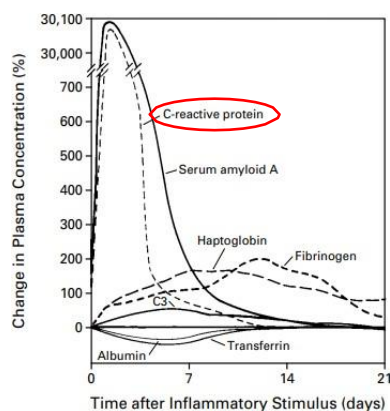
membrane.<sup>36</sup>

Induction of CRP gene transcription primarily occurs in hepatocytes in the liver in response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6) and tumor necrosis factor (TNF- $\alpha$ ).<sup>37</sup> In hepatocytes, pentameric proteins are retained in the endoplasmic reticulum by binding to two carboxylesterases, gp60a and gp50b.<sup>38</sup> When in a resting state (non-inflammatory), CRP works by being released slowly from the endoplasmic reticulum, following an increase in inflammatory cytokine levels, where if inflammation occurs, the binding of CRP to carboxylesterase is reduced/decreased, and CRP will be secreted quickly.<sup>27</sup> Stimulation of CRP synthesis occurs primarily in response to pro-inflammatory cytokines, especially IL-6 and to a lesser extent IL-1 and tumour necrosis alpha (TNF- $\alpha$ ).<sup>39</sup>

CRP shows increased expression during inflammatory conditions such as rheumatoid arthritis, some cardiovascular diseases, and increases many times in infectious conditions.<sup>27</sup> Elevated levels of CRP have been found in patients with appendicitis, cholecystitis, pancreatitis, and meningitis.<sup>40</sup> In patients suffering from symptoms of acute appendicitis, the possibility can be ruled out if the CRP level in the blood is <25 mg/L taken 12 hours after the onset of symptoms.<sup>41</sup> When

clinical symptoms of cholecystitis occur together with a CRP level of more than 30 mg/L, Accurate diagnosis of cholecystitis can be obtained with a sensitivity of 78%, indicating that CRP is a more sensitive marker than erythrocyte sedimentation rate and white blood cell count in supporting the diagnosis of cholecystitis.<sup>42</sup> In acute pancreatitis, a CRP level of more than 210 mg/L is able to differentiate between mild and severe cases, with a sensitivity of 83% and a specificity of 85%.<sup>43</sup>

As an acute phase protein, plasma concentrations of CRP increase by at least 25% during the presence of inflammation.<sup>44</sup> The highest concentrations of CRP are found in serum; with bacterial infections, CRP levels can increase many-fold.<sup>45</sup> Plasma CRP levels increase from approximately 1  $\mu\text{g}/\text{mL}$  becomes more than 500  $\mu\text{g}/\text{mL}$  within 24- 72 hours after severe tissue damage such as in trauma and cancer conditions (Figure 3).<sup>19</sup> However, when a stimulus ends, the CRP value will decrease exponentially in the range of 18- 20 hours to levels normal.<sup>46</sup> IL-6 is an inflammatory mediator as the main inducer of CRP gene expression, together with IL-1 $\beta$  will increase its inflammatory effect.<sup>20</sup> Although IL-6 is required for induction of the CRP gene<sup>47</sup>, there are many factors that can changes in basal CRP levels, including age, gender, smoking status, body weight, lipid levels, and blood pressure.<sup>31</sup>



**Figure 3. Characteristics of Changes in Acute Phase Plasma Protein Concentrations after Inflammatory Stimulus<sup>88</sup>**

Elevated CRP levels are not always related to disease, but can be caused by liver failure, which is one of the conditions that interferes with CRP production. There are only a few medical/drug therapies that can inhibit/reduce the increase in CRP levels, unless the drug treats the underlying pathological condition that causes the acute phase stimulus (underlying disease).<sup>48</sup> Several studies on oral hormone replacement hormone replacement therapy (HRT) cause The increase in circulating basal CRP levels becomes significant in postmenopausal women, which increases the risk of thrombotic events such as blood clotting conditions.<sup>49</sup> Ridker et al. found healthy postmenopausal women had a nearly twofold increase in circulating CRP levels when they took oral HRT and that CRP was the inflammatory marker most affected.<sup>50</sup>

A large body of research has confirmed that CRP is a predictive marker of cardiovascular disease and that use of HRT in postmenopausal women increases

the risk of stroke and blood clots.<sup>50,51,52,53</sup>

The majority of CRP research has focused on the role of CRP and its isoforms in cardiovascular disease and stroke. CRP is used as a strong independent predictor of cardiovascular disease in asymptomatic individuals.<sup>54</sup> CRP levels have been linked to prognosis in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease, and heart transplantation, suggesting that this has an active role in the pathophysiology of cardiovascular disease.<sup>55</sup> High sensitivity tests, such as nephelometric tests, are used to detect baseline CRP levels and patients at risk of cardiovascular disease. A person with a CRP level higher than 3 mg/L has an increased risk of coronary heart disease, and this risk is increased in those with type 2 diabetes.<sup>56,57</sup>

Although studies show that CRP levels increase during infection and inflammation, the precise role of CRP isoforms and the effects of each CRP isoform on specific cellular processes during disease progression are largely unknown. In addition to therapeutic strategies to inhibit CRP activity<sup>58</sup>, there are more targeted therapies for the treatment of CRP-mediated pathology, including inhibiting mCRP activity or preventing the dissociation of nCRP into mCRP.<sup>59,60</sup> Further studies are needed to be



able to identify and characterize the differences. the role of each CRP isoform at the site of local inflammation and infection and its relationship with other inflammatory cytokines.<sup>33</sup>

### **RELATIONSHIP BETWEEN GIVING POLYETHYLENE MICROPLASTICS AND INCREASING C-REACTIVE PROTEIN (CRP) LEVELS**

Microplastics (MPs) are toxic pollutants because they contain many dangerous chemicals. When microplastics and their toxic materials enter the human body through the ingestion route, various processes of microplastic absorption into the blood will occur. Microplastics with a size of up to 130  $\mu\text{m}$  will undergo paracellular absorption in intestinal epithelial cells, while microplastics with a size of  $<20 \mu\text{m}$  will undergo a phagocytosis process to reach the bloodstream. Toxic materials from microplastics in the bloodstream will cause an increase in the production of reactive oxygen species (ROS) and trigger oxidative stress reactions.<sup>10,20</sup>

The conversion of MPs to NPs increases the surface to mass ratio, allows them to pass through lipid membranes, and exacerbates the effects of intracellular toxicity.<sup>61</sup> Mitochondria as sites of the electron transport chain, oxidative phosphorylation, and ROS generation,

have been known to be the main site of ROS production due to exposure to MPs.<sup>62</sup> Furthermore, Lin et al., reported that NPs were able to damage mitochondria in human liver (L02) and lung (BEAS-2B) cells, leading to decreased MMP, suppression of mitochondrial respiration, and disruption of metabolic pathways.<sup>63</sup> Moreover, due to their small size, there is a greater possibility that NPs will accumulate in mitochondria, which increases their ability to induce oxidative damage and apoptosis, compared with MPs.<sup>64,65</sup> Although MPs may not be able to penetrate mitochondria due to their large size, they are Major damage to the outer mitochondrial membrane can occur due to excessive oxidative stress and the opening of Na/K transmembrane channels.<sup>66</sup> Oxidative stress will then induce pro-inflammatory cytokines which will result in inflammation or injury to cells, tissues and organs.<sup>36</sup>

C-reactive protein (CRP) is the main mediator of the acute phase inflammatory response and is mainly synthesized by IL-6-dependent hepatic biosynthesis.<sup>67,68</sup> Interleukin-6 is synthesized in the early stages of inflammation and induces a number of acute phase proteins, including CRP.<sup>69</sup> There is a correlation between increased IL-6 levels during inflammation and increased CRP levels<sup>20</sup>, with IL-6 inducing the CRP gene.<sup>47</sup> However, most

investigations of CRP production by IL-6 have generally failed to demonstrate which type of CRP isoform is produced. In some previous studies, antibodies have been used to identify that nCRP plays an important role, but considering that IL-6 occurs at the site of inflammation, pentameric CRP may have dissociated to mCRP.<sup>33</sup>

The primary role of CRP in inflammation tends to focus around the activation of the C1q molecule in the complement pathway leading to pathogen opsonization. Although CRP can initiate the body's fluid phase defense pathway by activating the complement pathway, it can also initiate cell-mediated pathways by activating complement and binding to IgG Fc receptors.<sup>67</sup> CRP binds to Fc receptors with the resulting interaction leading to the release of pro-inflammatory cytokines. CRP also has the ability to recognize self (host) and foreign molecules based on pattern recognition, complement activators such as IgG cannot achieve this because these molecules only recognize certain different antigenic epitopes.<sup>70</sup>

CRP is naturally deposited at sites of inflammation and sites of tissue damage.<sup>71</sup> Literature shows that CRP binds to damaged cell membranes and contributes to the inflammatory response<sup>72</sup>, with CRP molecules becoming associated with terminal complement complexes, especially in atherosclerotic lesions.<sup>73</sup>

Lagrang et al. provided evidence that CRP localizes to infarcted heart tissue and promotes local complement activation, triggering further damage to cardiac tissue.<sup>74</sup> Gitlin et al. concluded that CRP is localized to the nuclei of cells within the synovium of rheumatoid arthritis patients, but the cell type was not identified at that time.<sup>75</sup> However, other studies have shown no significant localization of CRP in a number of pathologies, thus suggesting that CRP is found primarily in the fluid phase rather than being deposited in tissue at the site of inflammation or injury. Little research has been conducted on the localization of CRP in inflammatory cells to date.<sup>76</sup>

Evidence shows that CRP is not just a marker of inflammation (biomarker) but also plays an active role in the inflammatory process. However, most of the early studies in the literature only referred to CRP and did not differentiate between the two isoforms. Thus, unlike more recent publications, findings from early studies of CRP may appear somewhat unclear and sometimes contradictory since it was often not specified which CRP isoform was measured or used in the experiment, whether the response was attributed to nCRP, or may actually be caused by partial dissociation.

## METHOD

This research is experimental analytical research. This research is experimental research, because the measurement/collection of quantitative data or information was carried out at two times (pre and post) (Figure 4) and the researcher intervened on the population and sample (*Rattus norvegicus* Strain Wistar). The quantitative data collected was then entered into the SPSS 22 program, for further comparative analysis (pre-post-test control group design) between C-Reactive Protein (CRP) levels in the blood serum of Wistar Strain *Rattus norvegicus* before and after being given polyethylene microplastic intake.

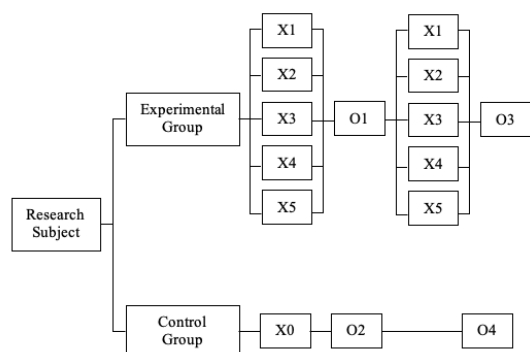


Figure 4. Pre-Post Test Control Group Design Research Model

The sampling technique is random allocation/simple random sampling, namely giving each rat a random number, then the researcher will draw each number to put the rats into 5 groups (6 experimental groups and 1 control group). Before both groups were given treatment, a pre-experimental c-reactive protein examination was carried out first using a vein puncture procedure,

namely taking blood through the v. Saphena Magna *Rattus norvegicus* Wistar Strain. The collected blood was examined for serum c-reactive protein levels using the ELISA method at the Institute of Tropical Diseases Research Center, Airlangga University.

Next is the stage of administering microplastic doses to 5 experimental groups with respective doses of polyethylene microplastic particles as follows: X1 = 0.0375 mg/day; X2 = 0.075 mg/day; X3 = 0.15 mg/day; X4 = 0.3 mg/day; and X5 = 0.6 mg/day dissolved in 1ml Aquabides, while the control group (X0) was only given 1ml Aquabides without polyethylene microplastics. During the research process, the Wistar Strain *Rattus norvegicus* was kept and cared for routinely in Animal Laboratory animal cages, every day given treatment according to the respective dose via oral probe. Treatment This research was carried out for 90 days.

After 90 days, *Rattus norvegicus* Wistar strain blood was taken again using a cardiac puncture procedure (ventricular blood). Previously, *Rattus norvegicus* Wistar strain was anesthetized with a mixture of ketamine + xylazine injection of 5-10 mg/kgBW. 2-3 cc of blood was taken and collected in a plain Vacutainer sample tube which had been labeled for each treatment sample. After the blood was collected, the serum c-reactive protein level

was checked again using the ELISA method at the Institute of Tropical Diseases Research Center, Airlangga University.

The next stage is statistical analysis and data exploration, namely after obtaining the data in the previous stage, proceed with identifying and assessing which risk factors are the cause of increased levels of c-reactive protein in the blood serum of *Rattus norvegicus* Wistar Strain. The data obtained was input into the SPSS 22 program and the data normality test was carried out using Shapiro-Wilk, and homogeneity of variance using the Levene test (normal and homogeneous if  $p > 0.05$ ) to see the homogeneity of the data obtained. Next, a statistical test was carried out using the two-way ANOVA test if the data was normally distributed or the Friedman test if the data was not normally distributed, to see the comparison of serum c-reactive protein levels before and after treatment to obtain the analysis results.

## RESULT

This research was carried out for approximately 90 days starting from 26 September 2023 to 26 December 2023 at the Animal Laboratory, Faculty of Pharmacy and Clinical Pathology Laboratory, Faculty of Medicine, Widya Mandala Catholic University, Surabaya, Pakuwon City, Surabaya as a place to care for experimental animals and check levels

of microplastic particles, as well as the Animal Laboratory of the Faculty of Veterinary Medicine and the Laboratory of the Institute of Tropical Disease, Airlangga University, Surabaya as a place for terminating experimental animals and checking serum CRP levels. The experimental animal population in this study was male Wistar Strain *Rattus norvegicus* with an age range of 2-3 months with a body weight range of 150-200 grams. The number of experimental animals used was 42, which were divided into 6 groups, namely 1 control group and 5 experimental groups, so that each group consisted of 7 experimental animals.

The results of the normality test using Shapiro-Wilk showed that the body weight data of the experimental animals in this study was normally distributed with a  $P$  value  $> 0.05$ . The results of the homogeneity test using Levene's Test showed a value of  $P = 0.561$  ( $P > 0.05$ ), so it can be concluded that the experimental animals used in this study came from the Wistar Strain homogeneous body weight. The results of the comparative test using One Way Anova showed a  $P$  value = 0.797, which means there was no significant difference in body weight between groups of experimental animals.

The results of the comparative test using the Friedman test showed a significant value of  $P = 0.000$  ( $P < 0.05$ ), so

it can be concluded that there is a difference in pre and post experimental CRP levels (Table 1). Based on the results of data processing using the SPSS application, the lowest mean result was 0.053, the highest mean was 0.405 with a standard deviation of  $\pm 0.117$  in the X0 group. In group X1, the lowest mean was 0.062, the highest mean was 0.535, with a standard deviation of  $\pm 0.202$ . In group X2, the lowest mean was 0.055, the highest mean was 0.792, with a standard deviation of  $\pm 0.317$ . In group X3, the lowest mean was 0.051, the highest mean was 0.955, with a standard deviation of  $\pm 0.426$ . In group X4, the lowest mean was 0.050, the highest mean was 0.973, with a standard deviation of  $\pm 0.430$ . In group X5, the lowest mean was 0.052, the highest mean was 0.980, with a standard deviation of  $\pm 0.432$  (Figure 5).

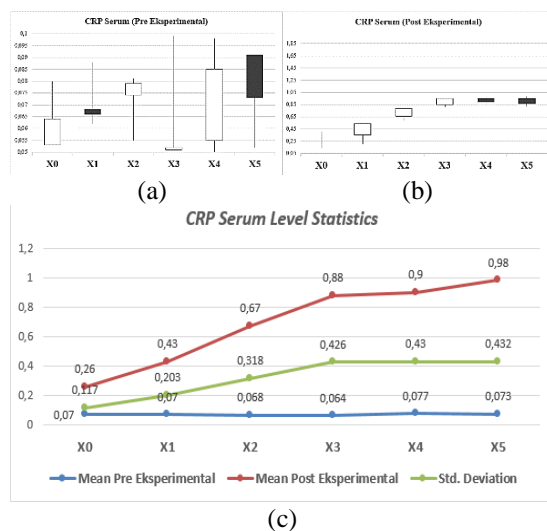


Figure 5. (a) Pre-Experimental Serum CRP Levels, (b) Post Experimental Serum CRP Levels, (c) Mean and Standard Deviation of Serum CRP Levels

Table 1. Comparative Test (Friedman Test)

Variable	Comparative test	P (<0.05)	Conclusion
Experimental Pre-Post Serum CRP Levels	Friedman Test	0.000	There is a significant relationship

## DISCUSSION

### Characteristics of Microplastics as Exposure Materials

This research was carried out to determine the effect of exposure to microplastics in different doses on serum CRP levels of Rattus norvegicus Wistar Strain in the control and experimental groups. The microplastic used in this research is Low Density Polyethylene (LDPE) microplastic which has a triangle marked with the number 4 on the packaging. LDPE was chosen as the exposure material because it is the type of plastic polymer that is currently most widely used in society, especially in the fields of construction, transportation and packaging products.<sup>78</sup>

Microplastics are toxic pollutants because they contain many dangerous chemicals. These chemicals are divided into 2 types, namely (1) plastic monomers and additives added in the plastic manufacturing process such as Bisphenol A (BPA), phthalates, nonylphenol, and (2) chemicals absorbed from the surrounding environment such as heavy metals.<sup>80</sup> When microplastics and their toxic materials enter the human body through the ingestion route, various processes of microplastic

absorption into the blood will occur. Microplastics with a size of up to 130  $\mu\text{m}$  will undergo paracellular absorption in intestinal epithelial cells, while microplastics with a size of  $\leq 20 \mu\text{m}$  will undergo a phagocytosis process to reach the bloodstream. Toxic materials from microplastics in the bloodstream will cause increased ROS production and oxidative stress reactions.<sup>81,82</sup>

Microplastics as exposure materials in this study were made to be  $\leq 20 \mu\text{m}$  in size, because this size is the size of microplastic particles that can be phagocytosed by M cells in Peyer's Patch, which is the main pathway for microplastic absorption into the blood. Microplastic particles are examined under a microscope before being exposed to experimental animals to determine their size. The microplastic powder used in this study had a diameter of between 4.2338  $\mu\text{m}$  to 18.634  $\mu\text{m}$  and was in the form of fragments with rough and sharp edges.<sup>(83,84)</sup> The increase in serum CRP levels from the control group to the X5 treatment group may be due to increased production of Reactive Oxygen Species (ROS) as an oxidant, as well as oxidative stress mechanisms, especially in hepatocyte cells, smooth muscle cells, macrophage cells, endothelial cells, lymphocyte cells, and adipocyte cells that

induce the production of CRP proteins, especially native C-reactive protein. This is in line with research conducted by Deng et al. which stated that there was an increase in ROS and oxidative stress in Wistar Strain *Rattus norvegicus* exposed to polystyrene microplastics with sizes of 5  $\mu\text{m}$  and 20  $\mu\text{m}$ . Induction of CRP gene transcription which occurs mainly by hepatocytes in the liver is a response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6) and tumour necrosis factor (TNF- $\alpha$ ) due to inflammatory or pathological processes at the cellular level. As an acute phase protein, plasma CRP concentrations increase by at least 25% during inflammation. Plasma CRP concentrations increase from approximately 1  $\mu\text{g/mL}$  to more than 500  $\mu\text{g/mL}$  within 24-72 hours after severe tissue damage occurs as in trauma and cancer conditions (normal values in white mice 300-600 ng/mL). In this study, there was a significant difference in serum CRP levels ( $P = 0.000$ ) from the results of the Friedman comparative test, where there was an increase in serum CRP levels after treatment (post experimental) from the lowest mean of 0.05 mg/L (pre-experimental) to the highest of 0.98 mg/L on average. This shows that administering microplastic intake at the dose studied had an impact on increasing serum CRP levels

in response to an inflammatory response.

### **Analysis of Experimental Pre-Post Serum CRP Level Examination Results**

The results obtained showed that initially there was an increase in serum CRP levels, namely from an average of  $0.16 \pm 0.12$  mg/L in the control group (X0), to  $0.25 \pm 0.20$  mg/L in the treatment group X1 (MP dose 0.0375 mg/day),  $0.37 \pm 0.32$  mg/L in treatment group X2 (MP dose 0.075 mg/day),  $0.44 \pm 0.43$  mg/L in treatment group MP 0.15 mg/day),  $0.46 \pm 0.43$  mg/L in treatment group X4 (MP dose 0.3 mg/day), and the highest peak level of  $0.49 \pm 0.43$  mg/L was found in the X5 treatment group (MP dose 0.6 mg/day). Tests for normality and homogeneity of the data were carried out at the next stage, and it was found that the data was distributed non-normally and homogeneously. Because the data was not normally distributed, a comparison test was carried out using the non-parametric Friedman test and obtained significant results with a value of  $P = 0.000$  ( $P < 0.05$ ), which means there was a significant difference in pre- and post-experimental serum CRP levels. between groups.

The increase in serum CRP levels from the control group to the X5 treatment group may be due to increased production of Reactive Oxygen Species (ROS) as an oxidant, as well as oxidative stress mechanisms, especially in hepatocyte

cells, smooth muscle cells, macrophage cells, endothelial cells, lymphocyte cells, and adipocyte cells that induce the production of CRP proteins, especially native C-reactive protein (nCRP).<sup>37,85,86</sup> This is in line with research conducted by Deng et al. which stated that there was an increase in ROS and oxidative stress in Wistar Strain Rattus norvegicus exposed to polystyrene microplastics with sizes of 5  $\mu\text{m}$  and 20  $\mu\text{m}$ . Induction of CRP gene transcription which occurs mainly by hepatocytes in the liver is a response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6) and tumor necrosis factor (TNF- $\alpha$ ) due to inflammatory or pathological processes at the cellular level.<sup>87</sup>

As an acute phase protein, plasma CRP concentrations increase by at least 25% during inflammation.<sup>44</sup> Plasma CRP concentration increase from approximately 1  $\mu\text{g/mL}$  to more than 500  $\mu\text{g/mL}$  within 24-72 hours after severe tissue damage occurs as in trauma and cancer conditions (normal values in white mice 300-600 ng/mL).<sup>19</sup> In this study, there was a significant difference in serum CRP levels ( $P = 0.000$ ) from the results of the Friedman comparative test, where there was an increase in serum CRP levels after treatment (post experimental) from the lowest mean of 0.05 mg/L (pre experimental) to the highest of 0.98 mg/L

on average. This shows that administering microplastic intake at the dose studied had an impact on increasing serum CRP levels in response to an inflammatory response.

Microplastic particles that are ingested into the gastrointestinal tract of mice will experience an endocytosis mechanism by M cells in intestinal lymphoid tissue, namely Peyer's patches, so that they are absorbed into the bloodstream.<sup>10,11</sup> This then triggers an increase in reactive oxygen species (ROS) which causes oxidative stress. In acute and chronic conditions, oxidative stress will induce pro-inflammatory cytokines which will result in inflammation or injury to cells, tissues and organs, one of which is endothelial dysfunction.<sup>10,85,86</sup> Interleukin-6 (IL-6) is an inflammatory mediator as the main inducer of CRP gene expression, together with interleukin-1 (IL-1) which will increase its inflammatory effect.<sup>20</sup> Although IL-6 is required for the induction of the CRP gene<sup>47</sup>, there are many factors that can change basal CRP levels include age, gender, smoking status, body weight, lipid levels and blood pressure.<sup>31</sup> Elevated CRP levels are not always related to disease, but can be caused by liver failure, which is one a condition that interferes with CRP production.

The majority of CRP research focuses on the role of CRP and its isoforms in cardiovascular disease and stroke alone.

Although many studies show that CRP levels increase during infection and inflammation, the exact role of CRP isoforms and the effects of each CRP isoform on specific cellular processes during disease progression are unknown. Most of the early studies in the literature only referred to CRP but did not differentiate between the two isoforms. Further studies are needed to identify and characterize

The limitation of this research is that there are no previous journals that have examined the effect of microplastic administration on serum CRP levels, resulting in a lack of research that can be used as a reference or comparative theory. In this study, the levels of microplastic particles in food and drinks given to experimental animals were not examined, so that confounding factors from food and drinks that might be contaminated by microplastics were not considered. The dose of microplastics in this study was not based on the body weight of the experimental animals, so it is not yet known whether variations in the body weight of the experimental animals affected the results of microplastic particle levels in the blood or serum CRP levels.

## CONCLUSION

Based on the results of this research, administration of Microplastic



polyethylene at the dose studied in the treatment group caused a significant increase in *Rattus Norvegicus* Wistar Strain Serum CRP levels.

There are several suggestions from researchers that can be considered for future research, including: research such as multivariate analysis to explain the impact of variations in subject characteristics on the increase in serum CRP levels. Recommending that in future research, food and drink given to experimental animals be examined, because they also have the potential to be contaminated with microplastics.

Microplastic dosage calculations be carried out according to the body weight of each rat so that the rat's body weight does not become a confounding factor in the research results. And finally recommends that future research be able to identify and characterize the different roles of each CRP isoform, especially nCRP and mCRP at local inflammation/infection sites as well as their relationship with other inflammatory cytokines. So that more accurate data is obtained.

## REFERENCES

1. Wright SL, Kelly FJ. Plastic and Human Health: A micro issue. *Environ Sci Technol.* 2017;51(12):6634–47.
2. Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. Plastic waste inputs from land into the ocean. *Science.* 2015;347(6223):768–71.
3. Diaz-Basantes MF, Conesa JA, Fullana A. Microplastics in honey, beer, milk and refreshments in Ecuador as emerging contaminant. *Sustainability.* 2020;12(14):5514.
4. Lee H, Kunz A, Shim WJ, Walther BA. Microplastic contamination of table salts from Taiwan, including a global review. *Sci Rep.* 2019;9(1):10145.
5. Ribeiro F, Okoffo ED, O'Brien JW, Fraissinet-Tachet S, O'Brien S, Gallen M, et al. Quantitative analysis of selected plastics in high-commercial-value Australian seafood by pyrolysis gas chromatography mass spectrometry. *Environ Sci Technol.* 2020;54(15):9408–17.
6. Oliveri Conti G, Ferrante M, Banni M, Favara C, Nicolosi I, Cristaldi A, et al. Micro- and nano- plastics in edible fruit and vegetables. The first diet risks assessment for the general population. *Environ Res.* 2020;187:109677.
7. Cox KD, Covernton GA, Davies HL, Dower JF, Juanes F, Dudas SE.

- Human consumption of microplastics. *Environ Sci Technol.* 2019;53(12):7068–74.
8. Senathirajah K, Attwood S, Bhagwat G, Carbery M, Wilson S, Palanisami T. Estimation of the mass of microplastics ingested – A pivotal first step towards human health risk assessment. *J Hazard Mater.* 2021;404:124004.
  9. Toussaint B, Raffael B, Angers-Loustau A, Gilliland D, Kestens V, Petrillo M, et al. Review of micro- and nanoplastic contamination in the food chain. *Food Additives & Contaminants: Part A.* 2019;36(5):639–73.