

Research Article

Determine curcumin concentration in organ rats and in ovaries at ovarian cancer model rats using ultra performance liquid chromatography-mass spectrometry (MS)/MS

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ABSTRACT

Curcumin has several pharmacological effects including anti-inflammatory, reduce kidney damage, hepatoprotector, and inhibiting tumor growth in ovarian cancer. The use of curcumin is limited because it has low bioavailability and low capacity to reach target organs. This limitation is overcome by making it in the form of nanoparticles. This study aims to determine curcumin levels on some organ in healthy rat groups and on ovaries at ovarian cancer model rats that given curcumin and curcumin nanoparticles. Measurement of curcumin levels using UPLC-MS/MS. Determination of curcumin level in the organ was carried out in 2 treatment groups, group that received curcumin and group that received curcumin nanoparticles orally with a single dose of 100 mg/kg Body Weight (BW). At 180 minutes, rats were decapitated for liver, kidney and ovaries. The ovarian cancer rats were also divided into 2 groups and given curcumin and curcumin nanoparticles with repeated doses of 100 mg/kgBW/day and after 30 days, ovarian cancer rats were decapitated for the ovaries. In the group that received curcumin, the concentration of curcumin in the liver, kidney and ovaries were less than the detection limit, whereas in the group that received curcumin nanoparticles, the levels of curcumin are 1,866.6±22.60 ng/g in liver, 114.87±13.36 ng/g in kidney and 136.864±11.15 ng/g in ovaries. In ovarian cancer rats, levels of curcumin in the group receiving curcumin is 2.29±1.15 ng/g and 17.76±4.85 ng/g in the group receiving curcumin nanoparticles. Curcumin nanoparticles can increase the distribution of curcumin in liver, kidney, ovaries in healthy rats and in ovaries at ovarian cancer model rats.

1. INTRODUCTION

Curcumin, bis (4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione, is a hydrophobic polyphenol compound derived from the roots of the *Curcuma longa* plant. Curcumin is one of the largest components in curcuminoid compounds that are found in the roots of these plants^{1,2}. Curcumin has several pharmacological effects including antioxidant, anti-inflammatory, antifungal, antibacterial and anticancer³. As an antioxidant compound, curcumin can reduce or prevent various cell damage⁴. The anti-inflammatory activity of curcumin is shown by inhibition of several compounds involved

in inflammation, such as phospholipase, lipoxygenase, cyclooxygenase⁵. Curcumin also suppresses pro-inflammatory cytokines, TNF- α expression, and release which are mediated through regulation of gamma peroxisome proliferator-activated receptor (PPAR- γ). The anti-inflammatory effect of curcumin can be attributed to PPAR- γ . In that study, there was an increase in mRNA and PPAR- γ protein expression after curcumin was given, and the anti-inflammatory effect of curcumin was not seen when given the PPAR- γ antagonist⁶.

Many in-vitro studies on curcumin activity are carried out in ovarian cancer-sustainable cells. Inhibition of apoptotic proliferation and stimulation was seen in in-vitro studies on human ovarian cancer cells through increased expression of pro-apoptosis (bax) genes and decreased anti-apoptotic gene expression (bcl-2) in Ho-8910 sustainable cells and Zeshun Yu et al. in SKOV3 through cell inhibition inhibits the PI3K/Akt signal pathway which plays a role in cell growth, proliferation, and metabolism^{7,8}.

Curcumin is a hydrophobic compound that is practically insoluble in water⁹. Curcumin has a low bioavailability. This can be caused by low absorption of curcumin, high metabolism and the rapid elimination of the body. The low bioavailability of curcumin seen in several studies in experimental animals as well as in humans causes the use of curcumin to be limited in vivo. Preliminary studies related to the pharmacokinetics of curcumin carried out by Wahlstrom and Blennow using Sprague Dawley mice, showed that curcumin had poor absorption in the intestine where plasma levels of curcumin were not found after dosing of 1 g/kg BW¹⁰.

Curcumin has low absorption in the gastrointestinal tract due to the low solubility in water (0.03 μ M). The presence of a mucus barrier consisting of glycoprotein, water, lipids can block its diffusion from the lumen to the surface of the enterocyte. Mucus can bind to curcumin in a non-specific way both with protein and fat. The elimination of mucus using NAcCys as a mucolytic carried out by Berginc et al on mice can increase the absorption of curcumin. Although curcumin is lipophilic (log P>3), the permeability of curcumin in membranes is very dependent on pH where permeability increases in an acidic atmosphere¹¹.

Curcumin has poor bioavailability. The systemic bioavailability of curcumin is bad due to poor intestinal absorption. Most of the curcumin consumed will be removed with feces, only a small portion is absorbed and widely

converted to water-soluble metabolites, glucuronides, and sulfates which are then re-excreted. This situation limits curcumin to reach distant target organs¹².

Several attempts have been made to increase the absorption of curcumin, one of which is nanoparticles¹³. Drug delivery systems with nanoparticle technology have been used to overcome the bioavailability problems of therapeutic agents. Nanoparticles are very suitable for hydrophobic compounds such as curcumin. Drugs made in the form of nanoparticles generally will quickly diffuse through the tight junction gap to immediately circulate to a specific place or directly to the expected cell target¹⁴. In the form of curcumin nanoparticles, it is also known to increase cellular uptake, but its distribution to organs is not yet known¹⁵. Nanoparticles can increase penetration, increase plasma concentration, increase the bioavailability of curcumin, increase cellular permeability, protect curcumin from degradation and metabolism and will be absorbed in specific sites or target targets for desired drugs¹⁶.

Nanoparticles also influence molecular/drug distribution. This distribution is influenced by the ability of molecules to cross the tissue. Tissues that have gaps in the endothelial wall contribute to the distribution of particles, such as the liver, spleen, bone marrow and tumors. The speed of nanoparticle uptake is based on permeability and retention, also known as the Enhanced Permeability Retention (EPR) phenomenon and in tumor tissue, there is an increase in capillary permeability. Therefore, nanoparticles can increase the distribution of tissues or organs so that they can affect the efficacy and side effects of a drug in the form of nanoparticles.¹⁷ Nanoparticle curcumin is a drug delivery system that is widely used and is known to increase uptake into cells (cellular uptake)⁸.

This study was conducted to determine whether the formulation of nanoparticle curcumin by the encapsulation of chitosan using ionic gelation method can increase the absorption of curcumin so that the concentration of curcumin in the liver, kidney and ovarian tissues increases. This study also measured the levels of curcumin in ovarian cancer model rats that given nanoparticle curcumin and compared with unmodified curcumin. Nanoparticle curcumin is expected to be one of the additional therapeutic modalities as nephroprotector, hepatoprotector and in ovarian cancer therefore the survival of patients can increase significantly.

2. MATERIALS AND METHODS

2.1. Materials

Curcumin (PT. Plamed Green Science Limited, China, total curcuminoid content, $\geq 95\%$), CMC-Na, curcumin standard (Sigma, Aldrich), sodium tripolyphosphate (Brataco, Indonesia), chitosan (Sigma, Aldrich); glacial acetic acid P; P hydrochloric acid (HCl); dimethyl sulfoxide P (DMSO); 70% ethanol P; propylene glycol; tween 80; glycerin; Kolliphor EL (Sigma); potassium dihydrogen phosphate (KH₂PO₄); sodium chloride (NaCl); aqua destilata; aqua bidestilata; 7,12-dimethylbenz [a] anthracene (DMBA), Kalium hydrogen phosphate (K₂HPO₄), alcohol, betadine, sterile gauze, round cotton, silk thread 3-0, catgut, ketamine, xylazine, NaCl 0.9% (PT. Widatra Bakti), propanolol (as an internal standard), methyl tert-butyl ether / MTBE (Merck), 0.1% formic acid (Merck), Acetonitrile (Merck), Methanol (Merck).

2.2. Methods

2.2.1. Formulation of curcumin nanoparticles

The formulation of curcumin nanoparticles was carried out at the Faculty of Pharmacy at the Pancasila University. Chitosan polymer is made in 1% b/v in glacial acetic acid. 1 gram of powdered curcumin was dissolved in a solvent consisting of propylene glycol, ethanol, DMSO, tween, glycerin, and cremophore. Curcumin and chitosan solutions were mixed using a magnetic stirrer (magnetic stirrer) at a speed of 300 rpm at room temperature. Furthermore, it was dripped with 0.3% NaTPP in a 300 rpm magnetic stirrer to form nanoparticles.

2.2.2. Measurement of Curcumin levels in rats, kidneys and ovaries 3 hours after single oral preparation

This study was an in vivo experimental test in experimental animals (Wistar strain female rats) to determine the levels of curcumin in the liver, kidneys, and ovaries from the administration of chitosan-based curcumin and nanoparticle curcumin. This research was carried out after the Health Research Ethics Committee's obtaining approval from the Faculty of Medicine Universitas Indonesia (approval number: 1188/UN.2 F1/ETIK/2018).

Wistar strain female rats weighing 150-200 grams from the Laboratory of Research and Development of Biomedical and Basic Health Technology, Balitbangkes, Ministry of Health of the Republic of Indonesia. Animals are placed

in a room with constant temperature and humidity, adequate lighting, pellet food, and water ad libitum. The animals were divided into 2 treatment groups (n=5) randomly, the curcumin group (female rats given unmodified curcumin with dose 100 mg/kgBW) and the curcumin nanoparticles group (female rats given curcumin nanoparticles with dose 100 mg/kgBW). Three hours after oral administration, the rats were euthanasia for kidney, liver, and ovarian tissue.

2.2.3. Measurement of curcumin levels in ovaries at rats with ovarian cancer models after repeated oral administration for 30 days

An ovarian cancer model rats made by implantation of a thread coated with 7,12-dimethylbenz (a) anthracene (DMBA) on the ovary of the rat as a model of animal¹⁸ for testing curcumin levels in the ovary organs after oral repeated administration for 30 days. Approximately 2 cm of sterile silk suture 3-0 coated with DMBA (2 mg) implanted in ovarian tissue. The surgery begins with intraperitoneal administration using ketamine and xylazine. Surgery is performed on the retroperitoneal side, and the ovary is cleared of fat tissue. 3-0 silk suture coated with DMBA was implanted in ovarian tissue and the wound was again closed. After the surgical process, the rats are kept fed according to the conditions above. It takes at least 28 weeks from the implantation process to form a tumor period¹⁸. After 28 weeks, female rats from the Wistar strain who had received DMBA implantation were divided into 2 groups (i) The treatment group received unmodified curcumin (100mg/kg BW/day) for 30 days (ii) The treatment group received nanoparticle curcumin (100mg/kg BW/day) for 30 days. After administration of the test material for 1 month, the mouse was euthanized for ovarian organs.

2.2.4. Tissue samples

Organ/tissue samples (liver, kidney, and ovary) were washed with saline solution and then dried with filter paper and weighed. The ovarian homogenate is made by 100 mg of ovarian tissue in 1 ml of Normal Saline 0.9% using Ultraturax® homogenizer, then centrifuged at 12,000 rpm for 5 minutes at -4°C.

2.2.5. Validation and Measurement of curcumin levels in organs

Measuring the level of curcumin in the plasma and ovarian organs was carried out at PT. Farmalab Indoutama, Jakarta, Indonesia

uses LC-MS / MS (Waters). The analytical method and validation of the LC-MS/MS analysis of determining curcumin levels in biological samples follows European Medicine Agency (EMA) Guideline on bioanalytic method validation^{19,20}.

2.2.6. Sample stability

Sample stability test was conducted to evaluate the analyte stability in homogenate tissue samples under long-term (30 days) at -80°C. The test was performed using three replicates of low QC (3 ng/ml) and high QC (24 ng/ml) sample that are analyzed immediately after preparation and after the applied storage conditions for evaluation. Sample was considered to be stable if the mean value and CV at the level should be within $\pm 15\%$ for accuracy and precision²⁰.

2.2.7. Measurement of curcumin levels in liver, kidney and ovarian homogenates

Measurement of curcumin levels in homogenate using LC-MS/MS instruments. The LC-MS/MS system used was UPLC Waters and tandem mass spectrometry with a positive electrospray ionization source (ESI). Separation of analyte in the sample using a liquid chromatography system with Acquity UPLC®

BEH C18 column and mobile phase gradient in the form of 0.1% and acetonitrile formic acid with a ratio of 72 : 28 with analyte rate of 0.3 ml/minute and injection volume of 3 μ L. The retention time for curcumin and internal standards is in 2.5 and 1.6 minutes¹⁹. The mass spectrometric detector parameters were optimized and set as follow: argon desolvation as 350°C with a flow rate of 500 l/hour and capillary voltage of 3.5 V. During analyses, ESI parameters were performed in positive ion mode and set as follow: parent m/z and cone voltage are 369 and 34 V for curcumin analysis and 260 and 42 V for IS analyses; daughter m/z and collision energy are 177 and 22 V for curcumin analyses and 183 and 17 V for IS analyses¹⁹.

2.2.8. Standard and internal standard solution preparation

The standard solution calibration curve is made to the concentrations of 1, 2.5, 5, 10, 20 and 30 ng/mL. Sample quality control (QC) is made in 3 different concentrations, such as 3 ng/mL as Low QC, 15 ng/mL as Medium QC, and 24 ng/ml as High QC. Standard internal solution (propranolol) is made by dissolving in distilled water so that the concentration of 400 ng/ml is obtained²¹.

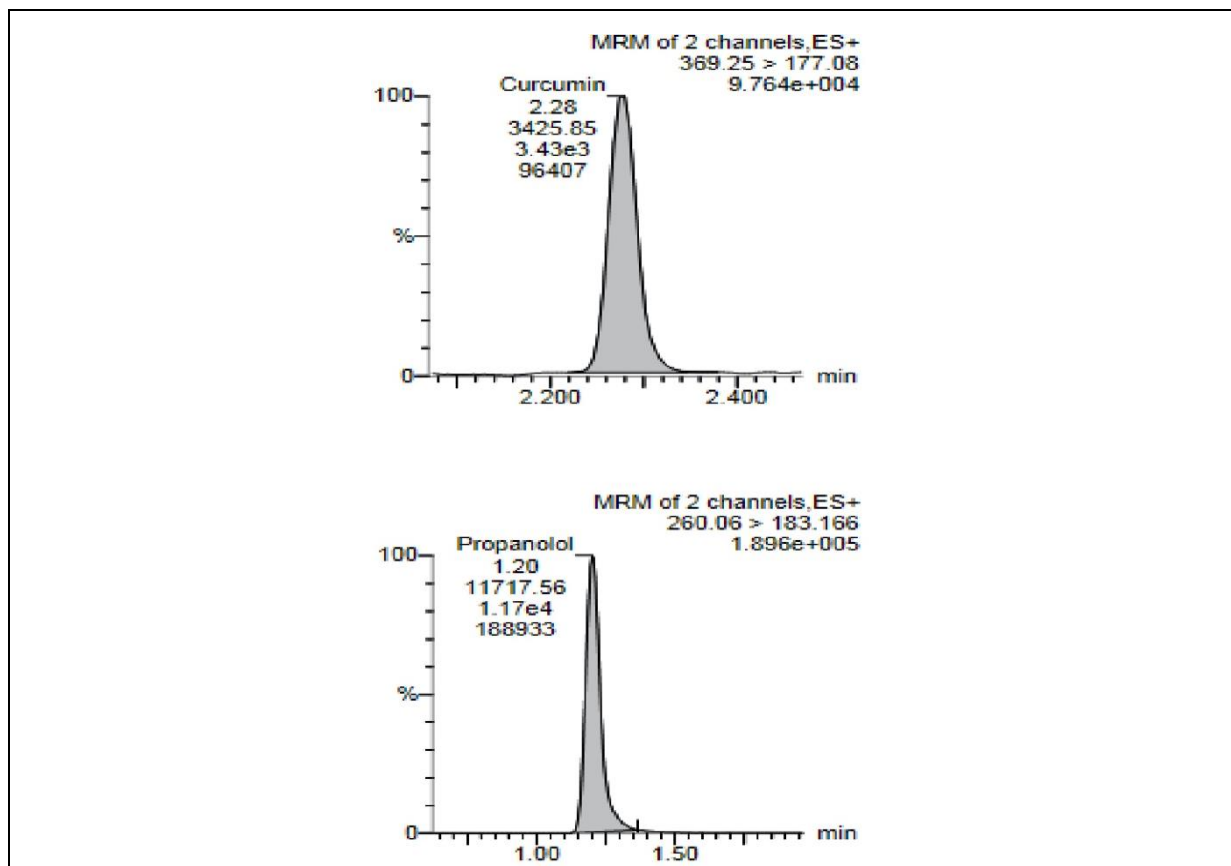


Figure 1. Representative chromatogram UPLC-MS/MS of Curcumin and Internal Standard in rat organ.

2.2.9. Sample Preparation

Two hundred μL of homogeneous samples (liver, kidney, ovary) containing curcumin was added 20 μL internal standard and 2 ml MTBE as extraction solution, then vortex for 1 minute and centrifuged at 3000 rpm for 10 minutes. The supernatant was transferred to the test tube and evaporated using an evaporator which was gassed with nitrogen gas at 600C for 2 minutes. The residue obtained was reconstituted with 100 μL of the mobile phase and vortex for 30 seconds. The solution was centrifuged at 12,000 rpm for 5 minutes and ready to use LC-MS/MS.

2.2.10. Calculation of levels in plasma and homogenates

The level of curcumin is obtained from the extrapolation ratio between the area of curcumin and the area of propranolol in the sample into the equation of the standard curcumin curve. The area of the analyte twice above the blank area is taken into account in the analysis.

2.2.11. Statistic analysis

Data are presented as the mean \pm SE. Each data was tested for the normality of its distribution with Shapiro Wilk and the homogeneity of its

variants with the Levene test. The Student's test was used for assessing unequal variance. A 2-tailed value of less than 0.05 was considered significant.

3. RESULT

In this study validation method of measuring curcumin levels in homogenate organs included accuracy, precision, and LLOQ (lower limit of quantification). The deviation of the accuracy value of the actual value of organ homogeneity was -7.86 to -10.65%. In organ homogenates, the coefficient of variation of precision was 3.04% -6.89%. In homogenate organs, the lowest level limit that can be measured is 1 ng / mL and the accuracy value obtained ranges from - 5.17% - 5.76% and the coefficient of variation is 9.14%. The value received by EMA is no more than 20% for the value of the deviation of the level and coefficient of variation²⁰. With the results of these validations, a method of measuring curcumin levels in homogenate organs can be used. The retention times of curcumin and IS were 2,28 and 1.20 minutes, respectively (Figure 1). The long-term sample stability data were shown in Table 1. They showed that the analyte was stable until 30 days due to their accuracy and precision in this condition.

Table 1. Accuracy and precision of long term stability in rat organ.

Nominal conc. (ng/ml)	Measured conc. (ng/ml) (mean \pm SD)	30 days	
		Mean accuracy (%)	Precision (%CV)
3.00	2.75 \pm 0.25	91.67	2.16
24.99	22.53 \pm 0.78	90.16	3.76

3.1. Levels of curcumin and nanoparticle curcumin in the liver, kidneys and ovaries of rats

Measuring the levels of curcumin and nanoparticle curcumin on liver homogenates after 3 hours of oral administration showed that nanoparticle curcumin levels were detected to be greater than the levels in the curcumin group. The concentration

level of curcumin in the liver, kidney, and ovarian homogenate in the group that received unmodified curcumin were less than the detection limit and the level of curcumin in liver, kidney, and ovarian homogenate in the group receiving nanoparticle curcumin were 1,866.60 \pm 226.01 ng/g; 114.87 \pm 13.36 ng/g; and 136.86 \pm 11.15 ng/g respectively. Levels and ratios can be seen in Table 2 and Figure 2.

Table 2. Levels of curcumin on liver, kidney and ovaries homogenate.

Organ	Measured conc. (ng/g)		Ratio
	Curcumin (n)	Curcumin nanoparticle (n)	
Liver	0.00 \pm 0.00 (5)	1,866.60 \pm 226.01 (5)	0 : 1,866.6
Kidney	0.00 \pm 0.00 (5)	114.87 \pm 13.36 (5)	0 : 114.8
Ovaries	0.00 \pm 0.00 (5)	136.86 \pm 11.15 (5)	0 : 136.8

Description: n = number of subjects detected, data is presented in mean values \pm SD

3.2. Levels of curcumin in ovaries at rats with ovarian cancer models

Levels of curcumin in homogenate ovaries rats with ovarian cancer after 30 days oral

administration of the sample at a dose of 100 mg/kg showed the level of curcumin in the group that received nanoparticle curcumin detected were greater than the levels in in the group that received unmodified curcumin. This can be seen in Table 3 and Figure 3.

Table 3. Levels of curcumin in ovarian homogenates cancer rats.

Sampling time (day)	Measured conc. (ng/g)		Ratio
	Curcumin (n)	Curcumin nanoparticles (n)	
30	2.288 ± 1.16 (5)	17.766 ± 4.85 (5)	1 : 7.76

Description: n = number of subjects detected, data is presented in mean values ± SD

4. DISCUSSION

Curcumin has been widely used in traditional medicine. Many studies have been conducted to find out the benefits and it was found that curcumin has several pharmacological effects including anti-inflammatory, antioxidant, antiviral, antifungal, antibacterial, immunomodulatory, and anticancer³. Curcumin can inhibit tumor growth, angiogenesis and induce apoptosis^{22,23}.

In the present study, level of curcumin in the liver, kidney, and ovarian homogenate in the group that received unmodified curcumin after 3 hours were less than the detection limit. The levels of curcumin in organ are found to be very low due to the high metabolism of curcumin in the body. This causes limited use of curcumin in vivo. Curcumin is metabolized into more polar compounds so that it can be excreted from the body. Curcumin undergoes metabolism both phase 1 and phase 2. In phase 1, curcumin

undergoes a double bond reduction in the structure of heptadione-3,5-dion which is catalyzed by NADPH-dependent curcumin/dihydrocurcumin reductase to tetrahydrocurcumin and hexahydrocurcumin which are widely formed metabolites, and reduced to small amounts of dihydrocurcumin and hexahydrocurcuminol. The enzymes involved in phase 1 are found in the cytosol of the liver and intestine. Also, another enzyme that plays a role in alcohol dehydrogenase. In phase II metabolism, curcumin and/or its metabolites will be conjugated with monoglucuronida or monosulfate which are catalyzed by the enzyme β -glucuronidase and sulfatase. The metabolites that are formed include curcumin glucuronide and curcumin sulfate which are found in the urine in the administration of oral curcumin, dihydrocurcumin glucorinda, tetrahydroxycurcumin (THC) glucuronide, hexahydrocurcumin (HHC) glucuronide which is found in bile^{24,25,26}.

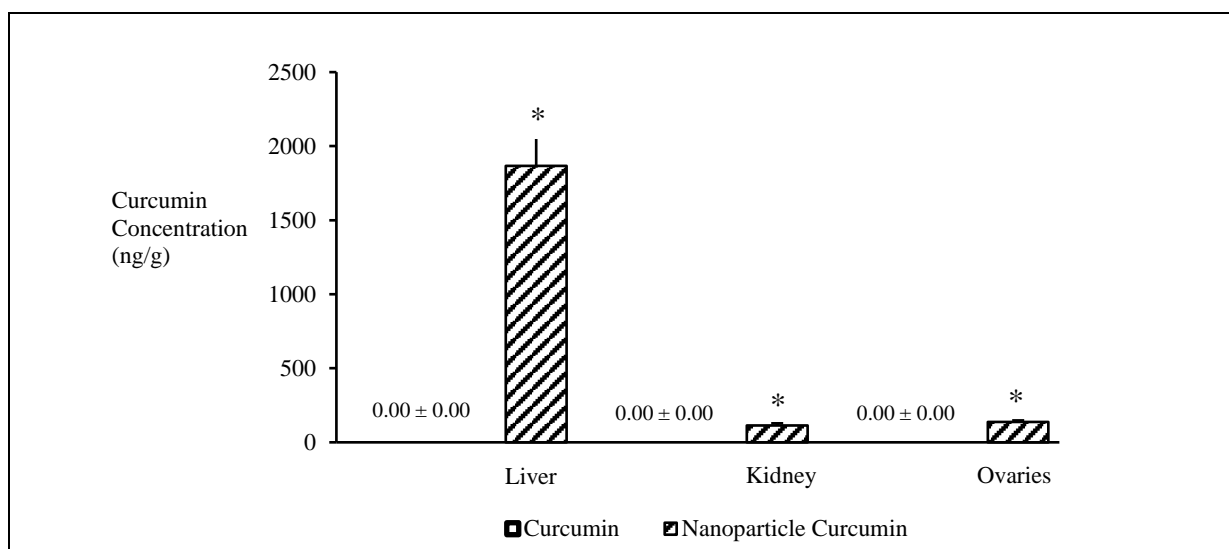


Figure 2. Curcumin level curve in rat liver, kidney and ovaries. The group given curcumin nanoparticles had higher levels of curcumin than the group given curcumin. These results were obtained 3 hours after administration of the test agent dose of 100mg/kgBW. (* $p < 0.05$ VS the curcumin group)

Holder et al. found that most of the curcumin metabolites were found in feces on oral or intraperitoneal administration. Giving labeled deuterium and tritium curcumin intravenously and intraperitoneally in mice showed that curcumin was excreted through bile with the

largest metabolites of THC and HHC glucuronide, and metabolite dihydroferulate and ferulic acid in small quantities²⁷. In addition, the enzyme UDP-glucuronosyltransferase (UGT) also plays a role in metabolizing curcumin^{25,28}. The distribution of curcumin in organs is very low. Ravichandran, 2013

conducted a study on the distribution of curcumin in several organs such as kidneys, liver, lungs, brain, and heart. The levels measured in the liver and kidneys were greater than the other organs with time to reach 90 minutes³⁹. Bharat B. Aggarwal, 2013 also reported the results of the distribution of curcumin at a dose of 340 mg/kg BW after 2 hours is very low⁴⁰. Jigni Mishra, 2017 did not get curcumin levels in the liver and kidneys after administering curcumin orally at a dose of 50mg/ kg BW in the 90th minute⁴¹.

In this study, we found the level of curcumin in liver, kidney, and ovarian homogenate in the group receiving nanoparticle curcumin were $1,866.60 \pm 226.01$ ng/g; 114.87 ± 13.36 ng/g; and 136.86 ± 11.15 ng/g respectively. Levels of curcumin in homogenate ovaries rats with ovarian cancer after 30 days oral administration of the sample at a dose of 100 mg/kgBW showed the level of curcumin in the group that received nanoparticle curcumin detected were greater than the levels in in the group that received unmodified curcumin. Metabolism and drug elimination can

also be affected by particle size. Large drugs/molecules are metabolized faster because the drug will be distributed to the liver in large numbers and recognized by macrophages that are widely found in liver tissue which will then be metabolized and eliminated from the body. This is known as the reticuloendothelial system (reticuloendothelial system / RES). Therefore, the nanoparticle formulation was developed to avoid RES by minimizing its bond to the protein so that it would increase the circulation time of the drug. Increased drug circulation time can be seen from the clearance (Cl) and half-life ($t_{1/2}$). The smaller the clearance and the greater the half-life of the meal, the circulation of the drug will be longer and so the amount of drug in the blood is large (AUC)¹⁷. Because nanoparticles can influence the process of absorption, distribution, metabolism, and elimination of drugs, the nanoparticles can influence the pharmacokinetic profile of the drug. Drugs that use a lot of this technology are generally drugs that have low solubility in water and/or low oral bioavailability^{14,29}.

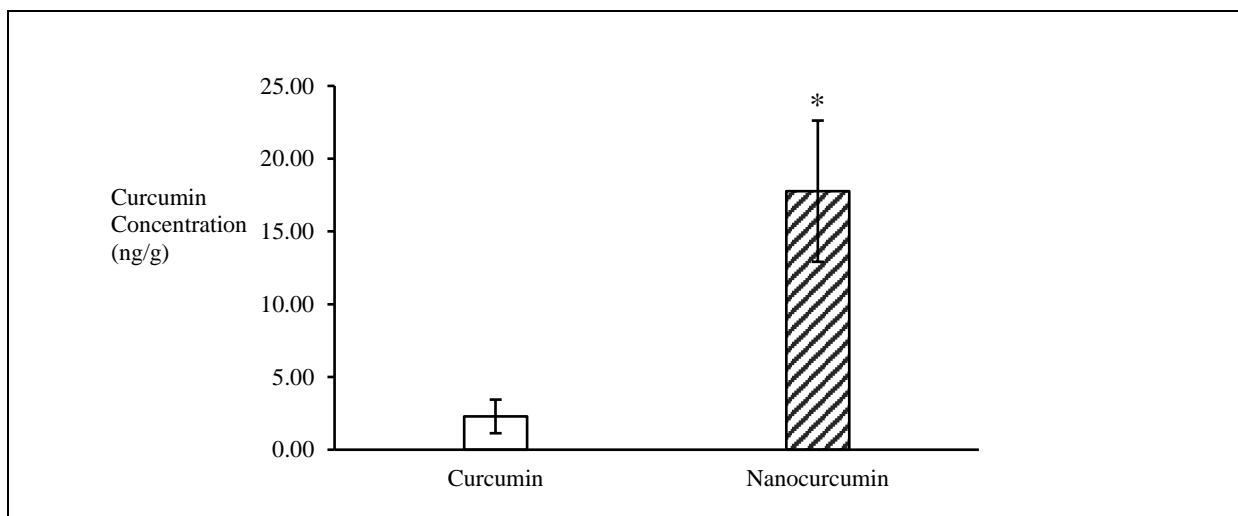


Figure 3. Curcumin level curve in ovarian cancer rats. The group given curcumin nanoparticles had higher levels of curcumin than the group given curcumin. This result was obtained 30 days after administration of the test agent dose of 100mg/kgBW. (* $p < 0.05$ VS the curcumin group)

Particle size also affects the transport process across the membrane and impacts the absorption process. Nanoscale particles/molecules (1-1,000 nm), especially with a range of 1-100 nm, can be transported through the mechanism of paracellular uptake, transcellular uptake by enterocytes, and transcellular uptake by M cells. Paracellular transport is transport through the gap between epithelial cells with disturbing the tight junction reversibly or through aquaporin in the gastrointestinal tract. Nanoparticles less than 20

nm can enter the circulatory system by reversing the tight junction. Nanoparticles with the use of certain polymers can also disturb the tight junction so that it increases paracellular transport³⁰. On transcellular nanoparticles (transcytosis) uptake, there are passive diffusion or involving receptors on cell surfaces that recognize nanoparticles specifically. The mechanism applies to transcellular uptake by enterocytes and M cells. M cells are more permeable than enterocytes so they can be targeted at micro delivery systems or nanoparticles³¹.

In this study, curcumin nanoparticles were made by ionic gelation method. In this method, cross-linking or beads occur, which is a bond formed between the polymers and the active site of the hydrophilic part which binds to the cross-linking agent to form structured and modified polymer strands.

The selection of crosslinking agents needs to be considered regarding stability, solubility, and pH when reacted together with the polymer to be used at optimum conditions so that it is possible to form stable synthesis results. Chitosan is an ideal polymer, has biodegradable properties and is biocompatible with other medicinal or excipient materials, such as non-toxic, inert and does not cause immune reactions to the body. Besides, it has swellable, edible and adhesive properties by forming a gel when it comes in contact with mucous fluid through its ionic bond. Chitosan can be applied for drug delivery both in microencapsulation systems, microspheres and in the manufacture of nanoparticle techniques. For this reason, this polymer is widely used as a mucoadhesive polymer^{32,33,34,35}. As a cross-linker, STPP was used in this study. STPP is inert, non-allergic, non-carcinogenic, biodegradable and biocompatible with the body. In general, STPP is used as a crosslinking agent or often referred to as a cross-linking agent for the synthesis of microparticles and nanoparticles with the polymer it reacts with. Variations in the use of concentration and a large amount of material used can affect the results of the formation of microparticles or nanoparticles. Therefore, optimization needs to be done first to require the desired synthesis^{36,37}. Chitosan is a positively charged (cationic) polymer reacted with a negatively charged (anionic) crosslinking agent so that the binding mechanism occurs ionically to form stable ions or molecules in the strands of the polymer chain. Tripolyphosphate (-PO₃H⁻) ion from sodium tripolyphosphate compound through its polyanion charge will interact with quaternary amine groups (-NH₃⁺) from chitosan polymers through polycation loads forming stable ionic bonds^{36,37,38}. With the nanoparticle formulation, curcumin can reach the target organ compared to unmodified curcumin. The encapsulation of curcumin with chitosan polymer protects curcumin from stage 1 and 2 RES and metabolism systems.

With the modification of particle size and the use of polymers can increase the distribution of curcumin in the organ and can reach the target organ. The submicron-sized particles are useful for drug delivery models with controlled-release targets as carriers that mediate the drug model so

that the drug directly works to the target^{14,29}. The form of curcumin nanoparticles in this study can increase the levels of curcumin in the liver, kidneys, and ovaries after being given orally in rats. In ovarian cancer model rats, there were also differences in the group that received nanoparticle curcumin and the group that received unmodified curcumin on repeated 30-day administration. This can provide information on curcumin formulations so that they can be developed and utilized in vivo and provide optimal pharmacological effects on the liver as hepatoprotection, kidney organs as renoprotection and use in ovarian cancer.

5. CONCLUSION

There are significant differences levels of curcumin from unmodified curcumin with curcumin nanoparticles in liver, kidney ovarian. The levels of curcumin in the form of nanoparticles are higher than unmodified curcumin in these organs, so the formulation of curcumin nanoparticles is a potential therapeutic agent such as hepatoprotection, organ kidney as renoprotection and use in ovarian cancer.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical approval from the Faculty of Medicine Universitas Indonesia (approval number: 1188/UN.2 F1/ETIK/2018).

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