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Biodistribution of ^{99m}Tc-HSA-nanoparticle in Lymphatic Node of Animal Model Induced by 7,12 Dimethylbenz(a)anthracene

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Abstract

Breast cancer is one of the most common malignant tumors found in women and has become as the second cause of death in women after cervical cancer. We successfully synthesized technetium-99m labeled Human Serum Albumin nanoparticle (^{99m}Tc-HSA nanoparticle); a radiopharmaceutical which potentially used for application in lymphoscintigraphy technique for tracing lymphatic system. This study was aims to obtain pre-clinical data from ^{99m}Tc-HSA nanocolloid in Sprague Dawley female rats given 7, 12-dimethylbenzeneanthracene (DMBA) - induced cell proliferation in the breast tissues. The rats are divided into two groups, one groups are given DMBA and observed for 5, 8 and 12 weeks and the other group is without any treatment as control. Biodistribution study of ^{99m}Tc-HSA nanoparticle was conducted to identify the accumulation of this agent in the popliteal and lumbar lymph nodes and the other tissue compare to normal rats. ^{99m}Tc-HSA nanoparticle shown good accumulation in popliteal lymph node 2.90 + 1.32 %ID; 0.81 + 0.47 %ID and 1.04 + 0.46 %ID at one hour post-injection in 5, 8 and 12 weeks respectively. The popliteal extraction (PE) was estimated with value 87.92 %, 74.07%, 71.79% in 5, 8 and 12 weeks respectively. This result showed that ^{99m}Tc-HSA nanoparticle is very promising compound to be lymphoscintigraphy agent in detection of breast cancer.
Keywords: lymphoscintigraphy, ^{99m}Tc-HSA nanoparticle, breast cancer

Biodistribusi ^{99m}Tc-HSA-nanopartikel pada kelenjar limfa hewan model yang diinduksi *7,12 Dimethylbenz(a)anthracene*

Abstrak

Kanker payudara merupakan salah satu tumor ganas yang paling umum ditemukan pada wanita dan menjadi penyebab kematian kedua pada wanita setelah kanker serviks. Nanopartikel *Human Serum Albumin* berlabel teknesium-99m (^{99m}Tc-HSA-nanopartikel) adalah radiofarmaka yang berhasil disintesis di PSTNT-Bandung yang berpotensi digunakan untuk melacak sistem limfatik dalam aplikasi teknik limfoskintigrafi. Penelitian ini bertujuan untuk memperoleh data pra-klinis dari ^{99m}Tc-HSA-nanopartikel pada tikus betina Sprague Dawley yang diberikan bahan karsinogenik *7,12-dimethylbenzeneanthracene* (DMBA) yang menginduksi proliferasi sel di jaringan payudara. Hewan coba dibagi menjadi dua kelompok dimana kelompok pertama diberi DMBA dan diamati masing-masing selama 5, 8 dan 12 minggu dan satu kelompok lainnya tanpa perlakuan sebagai kontrol. Studi biodistribusi ^{99m}Tc-HSA-nanopartikel dilakukan untuk mengidentifikasi akumulasi ^{99m}Tc-HSA-nanopartikel di kelenjar getah bening popliteal, lumbar dan jaringan lainnya dibandingkan dengan tikus normal. ^{99m}Tc-HSA-nanopartikel menunjukkan akumulasi yang baik di kelenjar getah bening popliteal 2.90 + 1.32 %ID; 0.81 + 0.47 %ID dan 1.04 + 0.46 %ID pada satu jam pascainjeksi masing-masing pada kelompok pengamatan hewan 5, 8 dan 12 minggu. Nilai ekstraksi popliteal (PE) pada masing-masing kelompok hewan uji 5, 8 dan 12 minggu adalah 87.92 %, 74.07%, 71.79%. Hasil ini menunjukkan bahwa ^{99m}Tc-HSA-nanopartikel merupakan senyawa yang berpotensi sebagai radiofarmaka untuk limfoskintigrafi dalam mendeteksi kanker payudara.
Kata kunci: limfoskintigrafi, ^{99m}Tc-HSA-nanopartikel, kanker payudara

1. Introduction

The prevalence of breast cancer in Indonesia is increasing and causing of death because it usually detected at an advanced stage. Most of breast cancer patients will survive if the cancer can be detected at the early stage. Therefore, accurate early detection is highly desirable so that appropriate therapy can be given before the primary tumors become widely spread.[1]

Lymphoscintigraphy is a technique in nuclear medicine to trace lymphatic vessels by injecting a colloidal radiopharmaceutical subcutaneous or intradermal which provides the results of scintigram imaging from the lymphatic system using a gamma camera.[2] In the case of breast cancer, the lymphoscintigraphy technique is very useful to identify the tendency of breast cancer become metastasized by observing the accumulation of radiopharmaceuticals at the first lymph node to receive lymphatic drainage from a tumor site, which is known as sentinel lymph nodes (SLN).[3-6] Several colloidal radiopharmaceuticals have been developed for lymphoscintigraphy and sentinel node localisation in breast cancer.[7,8] The other research also developed modification of radiopharmaceuticals for cancer sentinel nodes with bind to the mannose receptor.[9-11]

Human serum albumin-nanoparticles labeled with technetium-99m (^{99m}Tc-HSA nanoparticles) is a radiopharmaceuticals which has been successfully synthesized. This radiopharmaceutical made from the albumin protein with a form of nanometer spherical particles (100-200 nm) which has ideal particle size for lymphoscintigraphy.[12-16]

The study of administration 7,12-dimethylbenz(a)anthracene (DMBA) in animals shows that breast cancer will occur after passing through a series of administration of these compound. DMBA is a pro-oncogene compound that will be active after a metabolic process. The use of DMBA is based on biological properties which able to convert normal tissue into cancer tissue through the mechanism of free radicals.[17,18]

This study was conducted in order to determine of ^{99m}Tc-HSA nanoparticles accumulation in the popliteal and lumbar lymph nodes as a SLN model in animal model induced by DMBA. The results of this study are expected to present biological data of ^{99m}Tc-HSA nanoparticles that is very useful as radiopharmaceuticals for lymphoscinthigraphy and identify SLN in breast cancer.

2. Methods

The main material used in this study are human serum albumin (Sigma), glutaraldehyde (Sigma), sodium pyphosphate (E.Merck), $SnCl_2$ (WAKO) and [^{99m}Tc] Pertechnetate from ^{90}Mo/^{99m}Tc generators (Ansto) for preparation of ^{99m}Tc-HSA-nanoparticle.

7,12-dimethylbenz(a)anthracene (DMBA) (Sigma) as carcinogenic agent. Ketamine HCl (ketalar / Pfizer) and xylazine (seton 2% / Calier) are used for anesthesia procedures.

HSA nanoparticles was measured by Particle Size Analyzer (HORIBA). Radioactivity was measured using dose calibrator (Victoreen), TLC-scanner (AR-2000, BIOSCAN) and a Single Channel Analyzer with NaI(Tl) detector (Ortec, Model 4890)

2.1. Preparation of Animal Model

Animal studies were conducted in accordance with ethical approval of Ethics Committee for Care and Use of Experimental Animal - National Nuclear Energy Agency No: 003/KEPPHP/BATAN/V/2017. Animals were fed and housed in stainless steel cages in rooms with a temperature 25 ± 5 ° C, humidity of 35-60% and drank water *ad libitum*.

At first, 120 mg of DMBA was dissolved in carefully to 30 mL corn oil in centrifugation tube and homogenised by whirlimixer.

For the biodistribution study, 9 female Sprague-Dawley rats at 8 weeks of age administered DMBA orally 2 times a week for 5 weeks with a dose of 20 mg/kg BW and 3 others animals without any treatment as control. Animals were weighed weekly and beginning 5 weeks post-DMBA and palpation was carried out around the mammary breast

area to find out the presence of a tumor nodules.

2.2. Preparation HSA nanoparticles

HSA nanoparticle were prepared by a desolvation technique as described by Oekar *et al*. Briefly, 120 mg of HSA was dissolved into a glass vial with 1,2 mL of purified water. Then 4,8 mL of ethanol was added dropwise under stirring at room temperature until the solution become turbid. After the desolvation process, 44 µL of 2,5% glutaraldehyde solution was added to induced particle cross linking and purified water was added to a solution up to 8 mL. The process was continued under stirring of the suspension over a time period of 24 h. The resulting nanoparticle was heated for 2 hours at 70ºC, and centrifuged 2 times at 5000 rpm for 20 minutes. The precipitate formed is dispersed in 8 mL of purified water (stock solution). 100 µL of HSA-nanoparticles from stock solution was put into a glass vial and 900 µL of purified water was added then mixture was homogenize with whirlimixer and measures by Particle Size Analyzer

2.3. Radiolabeling of HSA-nanoparticles with technetium-99m

At first, 200 µL Sn-pyrophosphate solution (containing $SnCl_2$ 37,5 mg as reductor and 5 mg Na-pyrophosphate as co – ligand which diluted in 5 mL of water) was added into vial containing 50 µL HSA-nanoparticle. pH of the mixture was neutralized by addition of HCl 0,1 N then incubated for 15 minutes. Furthermore, 0,3-0,5 mL (1 mCi) of freshly eluted pertechnetate solution ($^{99m}TcO_4^-$) was added into the vial. The reaction mixture was mixed and incubated for other 15 minutes in room temperature.

2.4. Radiochemical yield determination of ^{99m}Tc-HSA nanoparticles

Radiochemical yield of ^{99m}Tc-HSA nanoparticles and other metabolites of ^{99m}Tc were determined by thin layer paper chromatography and cellulose acetate electrophoresis. Chromatography system are developed in ITLC-SG/ ethanol: water: ammonium mixture (2:5:1), Whatman 3 MM/ Methanol 95% and Whatman 3 MM/HCl.

2.5. In vivo studies

Biodistribution study of ^{99m}Tc-HSA nanoparticles was carried out on 9 animals administered DMBA and 3 others animals without any treatment DMBA. For the control 9 animals administered DMBA were divided into 3 groups of animals (n=3). First group was 5 weeks post-DMBA, second group 8 weeks post-DMBA and third group 12 weeks post-DMBA (rats that have formed breast cancer)

Animals were anesthetized with a mixture of ketamine HCl (dose 0,16 mL / 200 g BB) and xylazine 2% (dose 0,06 mL / 200 g BB) before the test. 20 □L of ^{99m}Tc-HSA nanoparticles carrying + 30 µCi of radioactivity was injected intradermally into left footpad of the animal. After injection, the footpad was massaged for 30 second. After 60 min injection, a 30 µL solution of the blue dye (patent blue V, SIGMA) in water was administered to the footpad. Ten minutes after the second injection, each animal was sacrificed by decapitation.[19,20] The popliteal and lumbar lymph nodes and tissues of interest were removed, weighed, and the radioactivity counts were determined with Single Channel Analyzer

The popliteal extraction (PE) was estimated according to the method as follows:[21]

PE = (%ID popliteal - %ID lumbar) - 100% (ID popliteal)

3. Result and Discussion

^{99m}Tc-HSA nanoparticles are radiopharmaceuticals made from human serum albumin in the form of nanometer which are dispersed in water for injection. As seen at Figure 1, on determination of particle size showed that of HSA-nanoparticle has an ideal particle size 100-200 nm for lymphoscintigraphy techniques.

Quality control of the ^{99m}Tc-HSA nanoparticles was assessed by thin layer paper chromatography to distinguish and quantify the amounts of radioactive contaminants (free $^{99m}TcO_4^-$, $^{99m}TcO_2$ and ^{99m}Tc-pyrophosphate).

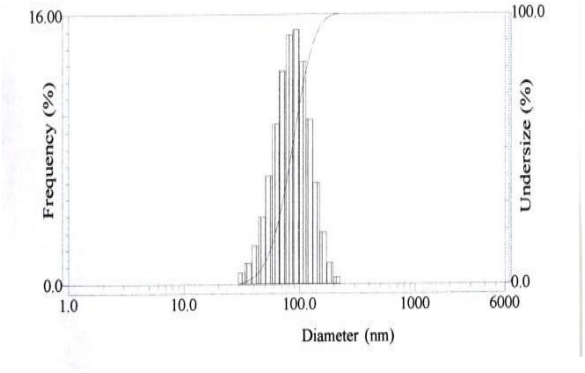

Figure 1. Particle size of HSA-nanoparticle

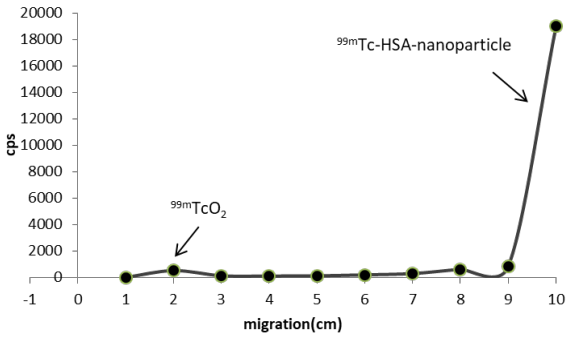

Figure 2. Chromatogram profile of ^{99m}Tc-HSA nanoparticles and TcO_2 with ITLC-SG/ Et-OH/H_2O/NH_3(2:5:1)

$^{99m}TcO_2$ was determined by using ITLC-SG/ ethanol: water: ammonium mixture (2:5:1) as the mobile phase where the $^{99m}TcO_2$ at the point of spotting while free ^{99m}Tc and ^{99m}Tc-HSA nanoparticles moved with the solvent front (Fig. 2). In Whatman 3 MM using methanol 95% as the solvent, free ^{99m}Tc moved with the solvent front, while ^{99m}Tc-HSA nanoparticles and $^{99m}TcO_2$ remained at the spotting point (Fig. 3). The remaining ^{99m}Tc-pyrophosphate as co-ligand was determined by Whatman 3 MM/HCl 1 N where ^{99m}Tc-pyrophosphate was found in Rf 0,5 and ^{99m}Tc-HSA nanoparticles moved with the solvent front (Fig. 4). Therefore, by calculating the free $^{99m}TcO_{4^-}$, $^{99m}TcO_2$ and ^{99m}Tc-pyrophosphate, labeling efficiency of the ^{99m}Tc-HSA nanoparticles could determine. ^{99m}Tc-HSA nanoparticles provide high radiochemical yield > 90% and can be used to carry out *in vivo* test.

In this experiment, the site specific carcinogen, DMBA was employed as an agent to induce mammary carcinoma in Sprague Dawley (SD) rats. The oral administration of DMBA activates cellular cytosolic receptor aryl hydrocarbon receptor (AhR). Activated AhR translocates into the nucleus and combines with AhR nuclear translocation protein. The AhR/ARNT complex induces gene transcription by binding to specific DNA recognition sites upstream to AhR responsive genes. This tumorigenesis involves AhR dependent up-regulation of cytochrome P450 (CYP1A1 and CYP1B1) enzymes, which metabolize DMBA into a mutagenic epoxide intermediate that forms DNA adducts. These DNA adducts are associated with mutations and the malignant transformation associated with polycyclic aromatic hydrocarbons (PAH)-mediated carcinogenesis.[17] This mechanism will form a malignant tumor in breast. When a malignant tumor occurs in breast on 12 weeks post-DMBA, a lymphatic fluid will flow to the sentinel lymph node

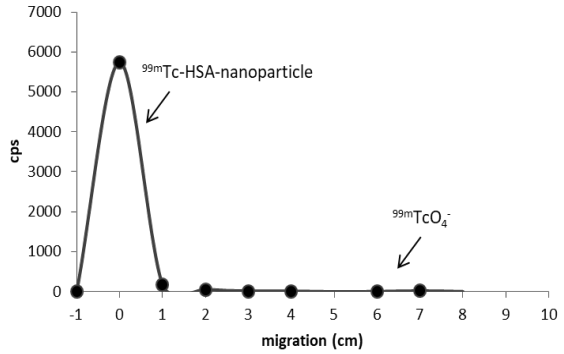

Figure 3. Chromatogram profile of ^{99m}Tc-HSA nanoparticles and TcO_{4^-} with Whatman 3 MM/Metanol 95%

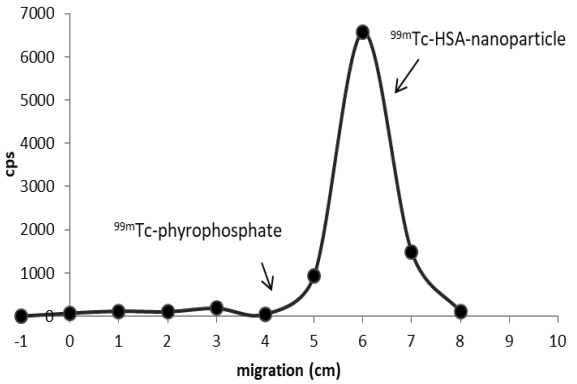

Figure 4. Chromatogram profile of ^{99m}Tc-HSA nanoparticles and ^{99m}Tc-pyrophosphate with Whatman 3 MM/HCl 1 N

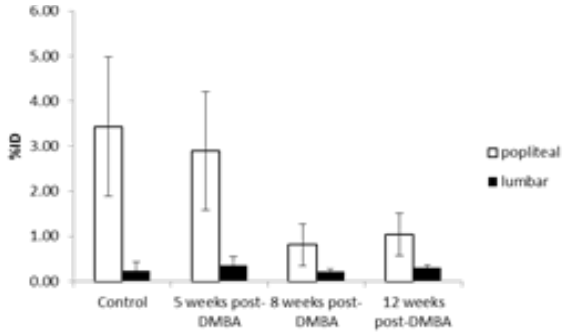

Figure 5. Biodistribution of radioactivity at 1 h post intradermal injection of ^{99m}Tc-HSA in popliteal and lumbar lymph node

(SLN) which defined as the first regional lymph node to receive tumour cells through the lymphatic system. [22] Sentinel node biopsy procedures was used in this model of breast cancer by injecting ^{99m}Tc-HSA nanoparticles a radioactive tracer that helps the surgeon to locate the sentinel nodes during experiment.

The radiopharmaceuticals for sentinel node detection should have rapid migration from injection sites to the sentinel node and prolonged retention of the radioactivity in the sentinel node.[23]

In this experiment we observed the accumulation of ^{99m}Tc-HSA nanoparticles in the popliteal and lumbar lymph nodes as a SLN model in 5, 8 and 12 weeks post DMBA administration. In this model breast cancer was developed to malignant with a presence of a tumor nodules after 12 weeks post administration of DMBA. ^{99m}Tc-HSA nanoparticle showed good accumulation in popliteal lymph node as sentinel node in this experimental model at one hour post-injection (Fig. 5). The radioactivity levels at the popliteal lymph node were decreased respectively at 5, 8, 12 weeks post DMBA administration when breast cancer developed to malignant compare to the control group. In the other hand, the radioactivity levels at the lumbar lymph node, the secondary lymph node in this model was increase with an increase of breast cancer.

Popliteal extraction (PE) is a parameter to estimate the selectivity of the radiopharmaceuticals to the sentinel lymph node. The high PE value means that the radiopharmaceutical was accumulated in the sentinel lymph node, and if the value of PE is low means the radiopharmaceutical could pass through sentinel node to secondary lymph node easily. The value of PE at 1 h post intradermal injection of ^{99m}Tc-HSA nanoparticles in control and 5, 8, 12 weeks post DMBA administration was shown on Table 1. The PE was estimated with value 87,92; 74,07; 71,79 on 5, 8, 12 weeks post DMBA administration respectively at 1 hour post injection while value PE of control animal was 93,40. This result indicated that ^{99m}Tc-HSA nanoparticle could pass through sentinel node to secondary lymph node in this animal model.

^{99m}Tc-HSA nanoparticle also showed radioactivity level in blood, liver and spleen at one hour post intradermal injection. These *in vivo* behaviors showed that ^{99m}Tc-HSA nanoparticle migrated from the injection site to peripheral lymph vessels, traveled through lymph node chain, and then emerged in the

Table 1. Biodistribution of ^{99m}Tc-HSA nanoparticles in rats at 1 h post-intradermal injection

Organ	Control	5 weeks post DMBA	8 weeks post DMBA	12 weeks post DMBA
Popliteal*	3.43 ± 1.54	2.90 ± 1.32	0.81 ± 0.47	1.04 ± 0.47
Lumbar*	0.23 ± 0.20	0.35 ± 0.20	0.21 ± 0.07	0.29 ± 0.06
Popliteal Extraction#	93.40	87.92	74.07	71.79
Blood^	2.38 ±0.71	2.38 ±0.40	0.63 ±0.46	1.01 ±0.15
Liver^	0.64 ±0.66	0.77 ±0.19	1.41 ±0.19	1.30 ±0.03
Spleen^	1.16 ±0.63	1.16 ±0.57	1.26 ±0.40	1.20 ±0.80

* Expressed as %ID
# (%ID popliteal-%ID lumbar) x 100 / (%ID popliteal)
^ Expressed as %ID/g

blood without being taken up directly into peripheral blood vessels. Since ^{99m}Tc-HSA nanoparticle was a colloidal particle, it might be recognize by macrophage in the liver and spleen. The increase of radioactivity ^{99m}Tc-HSA nanoparticles in the liver 5, 8, 12 weeks post DMBA administration compared to the control group because liver is the crucial organ responsible for detoxifying process, is shown to exhibit increased sensitivity to carcinogenesis induced by chemical carcinogens like DMBA, formation of breast cancer during administration of DMBA has metabolism in the liver and induce extensive liver damage carried out by oxidative stress and metabolism of DMBA by CYP450 enzyme.[18]

4. Conclusion

This study demonstrated that a ^{99m}Tc-HSA nanoparticles were exhibited accumulation in sentinel lymph node of breast cancer in animal model.

References

1. Departemen Kesehatan Indonesia. 2013. Laporan Hasil Riset Kesehatan Dasar (RISKESDAS) Nasional: Jakarta. Tersedia [Online]: http://www.depkes.go.id/resources/download/general/Hasil%20Riskesdas%2013.pdf
2. International Atomic Energy Agency. Radiopharmaceuticals for Sentinel Lymph Node Detection: Status and Trends. IAEA Hum Heal Ser. (6).Vienna:International Atomic Energy Agency;2015.
3. Rosso, K.J. and Nathanson, S.D. Techniques that accurately identify the sentinel lymph node in cancer. World J Surg Proced. 2015;5(1):14-26.
4. Núñez, E.G.F., Teodoro, R., Wiecek, D.P., Silva, N.G., Martinelli, J.R and Filho, R.S.O. Size and specificity of radiopharmaceuticals for sentinel lymph node detection. Acta Radiologica.2011;52:774-778.
5. Takeuchi, H., Kitajima, M., and Kitagawa, Y. Sentinel lymph node as a target of molecular diagnosis of lymphatic micrometastasis and local immunoresponse to malignant cells. Cancer Sci. 2008;99(3):441-450.
6. Devaki Shilpa Surasi, Janis O'Malley, and Pradeep Bhambhvani, 99mTc-Tilmanocept: A Novel Molecular Agent for Lymphatic Mapping and Sentinel Lymph Node Localization, J Nucl Med Technol 2015; 43:87–91
7. You-Jung Yang, Sung-Jig Li, Jeong-Yoon Song, Tc-99m Diphosphonate as a Potential Radiotracer to Detect Sentinel Lymph Nodes in Patients with Breast Cancer, Nucl Med Mol Imaging (2010) 44:62–68
8. G.M.M. Gommansa, E.Gommans F.M.vander Zant, G.J.J.Teule T.G.vander Schors, J.W.D.deWaard, 99mTc-Nanocoll : A radiopharmaceutical for sentinel node localisation in breast cancer - In vitro and in vivo results, Appl Radiat Isot. 2009 Sep;67(9):1550-8
9. Yamaguchi, A., Hanaoka, H., Pirmettis, I., Uehara, T., Tsushima, Y., Papadopoulus, M., and Arano, Y. Injection site radioactivity of 99mTc-labeled mannosylated dextran for sentinel lymph node mapping. Molecular Pharmaceutics.2015;12: 514-519.
10. Abul K. Azad, Murugesan V. S. Metz, Frederick O. Cope, Michael S. Blue, David R. Vera, and Larry S. Schlesinger, γ-Tilmanocept, a New Radiopharmaceutical Tracer for Cancer Sentinel Lymph Nodes, Binds to the Mannose Receptor (CD206), J Immunol 2015; 195:2019-2029;
11. Morais, M., Subramanian, S., Pandey, U.C., Samuel, G.V., Venkatesh, M., Martins, M.B., Pereira, S.M., Correia, J.D.G., and Santos, I. Mannosylated dextran derivatives labeled with fac-$[M(CO)_3]$+ (M = 99mTc, Re) for specific targeting of sentinel lymph node. Mol. Pharmaceutics. 2011;8:609-620.
12. Oekar, N.K., and Widyasari, E.M. Penandaan human serum albumin (HSA) nanospheres dengan radionuklida teknesium-99m. Majalah Farmasi Indonesia. 2008;19(3):117-127.
13. Sugiharti, R.J. Halimah, I., Widyasari,

E.M., and Kania, P.P. Biodistribusi 99mTc-human serum albumin-nanosfer pada mencit putih (Mus musculus) sebagai radiofarmaka untuk limfosintigrafi. Prosiding Seminar Nasional Sains dan Teknologi Nuklir; 2009 Juni 3; Bandung, Indonesia. Indonesia: PTNBR-BATAN; 2009.

14. Oekar, N.K. Pengembangan sediaan 99mTc-human serum albumin (HSA)-nanosfer sebagai radiofarmaka untuk limfosintigrafi. Prosiding Seminar Nasional Keselamatan Kesehatan dan Lingkungan VI; 2010 Juni 15-16; Jakarta, Indonesia. Indonesia: PTKMR-BATAN, FKM-UI, KEMENKES-RI; 2010.
15. Nanny Kartini Oekar, Eva Maria Widyasari, Epy Isabela, Karakteristik Fisiko-Kimia Radiofarmaka 99mTc-Human Serum Albumin (HSA)-Nanosfer, Jurnal Sains dan Teknologi Nuklir Indonesia, Vol. XI, No. 1, Februari 2010: 35-44
16. Eva Maria Widyasari, Nanny Kartini Oekar, Formulasi Kit Human Serum Albumin (HSA)-Nanosfer sebagai Limfosintigrafi di Kedokteran Nuklir, Jurnal Sains dan Teknologi Nuklir Indonesia Vol. 13, No.1, Februari 2012; 49 – 60
17. Kalyani Chowdary Karnama, Maheswara Ellutlaa, Lakshmi Narendra Boddulurua, Eshvendar Reddy Kasalaa,Shravan Kumar Uppulapua, Malayamarutham Kalyankumarrajua, Mangala Lahkara, Preventive effect of berberine against DMBA-induced breast cancer in female Sprague Dawley rats, Biomedicine & Pharmacotherapy 92 (2017) 207–214
18. Lakshmi Arivazhagan, Sorimuthu Pillai Subramanian, Tangeretin, a citrus flavonoid attenuates oxidative stress and protects hepatocellular architecture in rats with 7, 12 -dimethylbenz(a)anthracene induced experimental mammary carcinoma, Journal of Functional Foods 15 (2 0 1 5) 339–353
19. Renato Santos de Oliveira Filho, Geruza Rezende Paiva, Jairo Wagner, Solange Amorim Nogueira, Experimental model in rat for sentinel node biopsy1, Acta Cir Bras, 2003 Vol 18, Special Edition.
20. Pereira,R.J., Filho, R.S.O., Zuliani, C.R., and Pinheiro, L.G.P. Vital dye and carbon solution for sentinel lymph node biopsy in rats. Einstein. 2008;6(4):463 -466.
21. Kyoko Takagi, Tomoya Uehara, Emi Kaneko, Morio Nakayama, Mitsuru Koizumi, Keigo Endo, Yasushi Arano, 99mTc-labeled mannosyl-neoglycoalbumin for sentinel lymph node identification, Nuclear Medicine and Biology 31 (2004) 893–900
22. Cheng, M.H., Huang, J.J., Wu, C.W., Yang, C.Y., Lin, C.Y., Henry, S.L., and Kolios, L.K. The mechanism of vascularized lymph node transfer for lymphedema: Natural lymphaticovenous drainage. Plastic and Reconstructive Surgery.2014;133(2):192-198.
23. Derya İlem-Özdemira, Ulkem Yararbasb, Rajaram,Wendy L. Radiofarmaka untuk Studi Baha Zengelc, Gökhan Ertand, Makbule Asikoglua, Preparation of 99mTc-isosulfan blue for lymph node localization in rats, Applied Radiation and Isotopes 118 (2016) 338–342