Histology of Mice Skin Tissue Based on in Vivo Evaluation of the Anticancer Extracts of Marine Sponge *Aaptos Suberitoid*es

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***Abstract*—The sponge Aaptos suberitoides can produce high secondary metabolite with some farmocological activities as antimicrobial, antiviral, antiinflamatory, and anticancer agents. The purpose of this research is to find out correlations between activities of the etanol extracts of marine sponges Aaptos suberitoides on the cancer growth of subcutaneous mice (Mus musculus) injected by Benzo(a)piren. For the purpose the mice were divided into six groups, i.e. I, II, III, IV, V, and VI. Each group was treated with carcinogenic inductions by intravenously injecting the Benzo(a)piren concentration of 0.3g/0.2 ml oleum olivarum. After the cancer was appeared at the fifteenth day, the mice were treated by the anticancer extracts of marine sponges Aaptos suberitoides with concentrations of 500 mg/kg BB (Group IV), 1000 mg/kg BB (Group IV), and 1500 mg/kg BB (Group IV). The treatments were orally done each day for two weeks. At the twentieth week, subcutaneous cancer tissues were taken to make histological preparates using a parafin method. Result of the histological observation indicates that cancer in the mice was fibrosarcoma characterized by the thickening dermis layers, necrosis, mitosis, and nuclear polymorphsm. Necrosis, mitosis, and nuclear polymorphism occurred in Groups II, IV, V, and VI, and did not in Groups I and II. Presentation of necrosis was 20-60%, mitosis was in 3-4 cells, and nuclear polymorphism was 100%. Result of the statistical analyses by using the Kruskal-Wallis method and the Pair Comparison test indicates that the anticancer extracts of marine sponges with the concentrations of 500 mg/kg BB, 1000 mg/kg BB, and 1500 mg/kg BB had no activity inducing mice skin cancer.**

Keywords— **marine sponges, Aaptos Suberitoides, anticancer, fibrosarcoma**

***Abstrak*—*Penelitian ini dilakukan untuk mengetahui aktivitas ekstrak etanol spons Aaptos suberitoides terhadap sel kanker kulit mencit yang diinduksi benzo(a)pyren secara in vivo. Mencit (Mus musculus) dibagi ke dalam 6 kelompok yaitu kelompok I (mencit sehat), II (diberi CMC Na), III (diberi cyclophospamide), IV (ekstrak spons 500 mg/kg BB), V (ektsrak spons 1000 mg/kg BB) dan VI (ekstrak spons 1500 mg/kg BB). Induksi kanker dilakukan dengan pemberian Benzo(a)pyren selama 10 hari sebanyak 5 kali. Dosis 0,3 gram dalam 0,2 ml CMC Na). Pada minggu ke-20 mencit dikorbankan dan dilakukan pengambilan jaringan kanker pada kulit. Jaringan kanker dibuat preparat histologi dengan metode parafin dan dilakukan pengamatan histologi pada perbesaran 400 dan 1000X . Hasil pengamatan histologi didapatkan bahwa kanker pada mencit adalah fibrosarkoma, ditunjukkan dengan penebalan lapisan dermis, nekrosis, sel mitosis dan polimorfisme inti sel. Presentase nekrosis pada kelompok II dan kelompok terapi ekstrak spons (kelompok IV, V dan VI) berkisar antara 20-60%. Pada kelompok III dan I tidak ditemukan nekrosis. Jumlah sel mitosis pada kelompok II dan kelompok terapi ekstrak spons adalah 3-4 sel mitosis. Pada kelompok 1 dan III tidak ditemukan adanya sel mitosis. Persentase polimorfisme inti pada kelompok II dan kelompok terapi ekstrak spons adalah 100%. Sedangkan pada kelompok I dan III tidak ditemukan polimorfisme inti sel. Hasil analisa statistik metode Kruskal Wallis dan Uji Perbandingan Berganda, diketahui bahwa ekstrak spons pada konsentrasi 500 mg/kg BB, 1000 mg/kg BB dan 1500 mg/kg BB memiliki aktivitasantikanker yang rendah terhadap fibrosarcoma.*

Kata Kunci—***marine sponges, Aaptos Suberitoides, anticancer, fibrosarcoma***

I. INTRODUCTION

Indonesia is one of the countries with megadiversity in natural resources, particularly marine ones, because the country's territory is largely sea water area. The overwhelming biodiversity in natural resources makes the country having the nature-based comparative advantages to manage for people welfare. Some of the marine resources in the country's sea water area are fishes (Pelagic and demersal), algae, coral reefs, sponges, planktons, etc. However, to date, the resources are still unrecognized and largely not explored in an optimal manner. One of the resources is a sesil benthic organism with some farmocological activities as antimicrobial, antiviral, antiinflamatory, and anticancer agents [19].

One of the benthic organisms with high bioactive content is sponge. It can produce high secondary metabolite and has ability to synthesize various organic components such as polycetida, alkaloid, peptide, and terpene. The components can be used as medicine materials [20].

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The sponge *Aaptos Suberitoides* is a type of the sponges whose structure is a lobus globular osculiferus, brownish orange in color, with a rough surface and to be black in color if it was entered into alcohol [18]. It is recognized that it can produce a bioaktive compound of alkaloid, i.e. aaptamin containing 1H-benzo(de)-1, 6-napthyridine, and sitotoxic in nature. 1H-benzo(de)-1, 6-napthyridine is an important compound because it can retard the activity of CDK-Cyclin Complex. The CDK-Cyclin Complex is itself a complex of proteins with important role in abnormal cellular proliferation causing cancer [1].

The bioactive compound produced by the marine sponges is the sources of new compound with some farmacological activities, so it can be used as medicine material due to the toxic nature of killing any cancer cells [3]. The need for new medicine as anticancer compound is increasingly urgent, because the drugs currently used have the low level of effectiveness and expensive price. The search for new sources of the anticancer compounds is continuosly done to get bioactive compound resulted from marine organism such as sponge [15]. Prelimary study was conducted with aim of screening toxic compounds from several marine sponge species using the Brine Shrimp Lethality Test (BST) [2]. Two isolates that are toxic to larvae *Artemia* salina, i.e. the compound of marine sponge *Petrosia* sp, was successfully isolated and recognized to have a high level of toxicity to larvae *Artemia salina* with LC 50 of 7.23 µg/ml, dan to myeloma cell of 16.95 µg/ml. Based on the data of bioactive compounds obtained using the BST method and sitotoxicity test on cancer cell, it can be known that compounds toxic to larva *Artemia salina* are also toxic in nature to breast cancer cell (Astuti, *et. al.* 2005). It is recognized also that the bioactive compounds isolated from sponge have antibacterial activities due to the fact that the sponge species has ability to sythesize various organic compounds such as alkaloid, peptide, and terpene that are secondary metabolites. It is known also that the extracts of marine sponge diluted into polar solvents contain alkaloid, while those diluted into non-polar solvents contain alkaloid dan terpenoid [12].

Marine sponges collected from the sea water area of Pasir Putih Beach in Situbondo generally have bioactive compounds with potential as anticancer agent [11]. *Aaptos suberitoides* is the mostly toxic sponge with the LC50 value of 134.1362 ± 36.6114 ppm.

Bioactive compounds that are potentially used as anticancer agents should be tested firstly to experimental animal. Cytotoxicity test is one of the methods developed to predict the presence of compounds toxic to cell, which is absolutely required to be used as anticancer medicines [8].

The testing of the compound's activities as anticancer agent was started by inducing carcinogenic agent to experimental animals. One of the substances with carcinogenic effect is Benzo(a)pyren. The precarcinogen of Benzo(a)pyren will be altered to be active carcinogen through a process involving one or more enzymes-catalized reactions. Final carcinogen has highly reactive character and is generally an electrophyl that easily attacks nucleophylic entity (molecules rich of electrons) in DNA, RNA, and proteins. Chemical carcinogen has potential to attack cellular macromolecules, so it can result in severaly damaged DNA and genetic mutation [10].

Observation on anticarcinogenesis test can be done in a macroscopic and microscopic manner. The macroscopic probes of all groups are done on number of experimental animals with tumor, number of nodules of each tested animals, and the size of tumor. Based on the microscopic observation, description of the cancer cell is collected [9].

Histological changes in cancer tissue are characterized by the lack of tissue matrix, necrosis, no limit in cellular proliferation, and infiltrative to surrounding tissues, while cellular changes are characterized by a high ratio of cytoplasm to nucleus, a variation in nucleus and cytoplasm size, pleomorphism, big nucleolus, and a high rate of mitosis [14].

II. METHOD

Experimental animal used were 24 male mice (*Mus musculus*) of B Albino clone (BALB/c) strain with 3 months old, which were randomly divided into six groups. Before the treatment was done, all the mice were aclimated in the stable for a week and given the same feed and drinking water, i.e. Par G produced by Comfeed and Aquades.

A. Carsinogenic Induction

Carcinogenic induction by injecting Benzo(a)pyren was done at 2nd week in every two days for five times. Benzo(a)pyren of 0.3 gram was diluted in 0.2 ml of oleum olivarum and intravenously injected in subcutaneous cervix of the mice.

The mice was divided into six groups, including:

1. Group I : A control group with no treatment.
2. Group II : A placebo group subcutaneously injected by the oleum olivarium of 0.2 ml.
3. Group III : A treatment group induced by Benzo(a)pyren of 0.3 gram/ oleum olivarum of 0.2 ml.
4. Group IV : A treatment group induced by Benzo(a)pyren of 0.3 gram/ oleum olivarum of 0.2 ml.
5. Group V : A treatment group induced by Benzo(a)pyren of 0.3 gram/ oleum olivarum of 0.2 ml.
6. Group VI : A treatment group induced by Benzo(a)pyren of 0.3 gram/ oleum olivarum of 0.2 ml.

B. Test of Anticancer Extract of Marine Sponge Aaptos Suberitoides on Mice

After the cancer was appeared at 15 weeks, the treatment with anticancer extract of marine sponge *Aaptos suberitoides* was given orally for two weeks with concentrations of 0, 500 mg/kg, 1000 mg/kg, and 1500 mg/kg in weight of mice. The treatment group of mice were as follow:

1. Group I : A negative control group.

2. Group II : A control group administered by CMC Na of 0.2 ml (solvents of the extract of marine sponge *Aaptos suberitoides*).
3. Group III : A positive control group (placebo) administered by a anticancer drug of cyclophospamide of 0.4 mg/CMC Na of 0.2 ml.
4. Group IV : A treatment group administered by sponge extract of 500 mg/kg.
5. Group V : A treatment group administered by sponge extract of 1000 mg/kg.
6. Group VI : A treatment group administered by sponge extract of 1500 mg/kg.

Fibrosarcoma tissue were taken at 17th week or two weeks after treatment using the extract of marine sponge *Aaptos suberitoides* was given. The mice were killed in an eutanasia manner by using chloroform and then the cancer tissue were taken. The histological section of mice cancer tissue was made by using a parafin method.

C. Parafin Method

The observation of cancer tissue made by parafin method. The prosedures of preparat tissue and organ were showed i.e.

1. Fixative. The cancer tissue moved in fixative solvent formalin 40% for time 24 hours.
2. Dehidration. The cancer tissue moved in fixative alcohol 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 96% Each 20 menit submersion of fixative alcohol.
3. Clearing. The cancer tissue moved in fixative alcohol-xylol for time 40 minute. Then, their moved in pure xylol I, II, III each 20 minute.
4. Infiltrasi. The cancer tissue moved in liquid toluol-paraffin cair for time 20 minute. The paraffin I, II and III were moved into oven by 56˚C temperature.
5. Embedding. The liquid paraffin enter to mold until full. The piece of organs have entered to liquid paraffin to massive. The position of tissue in liquid paraffin were crossed of the bottom.
6. Sectioning. The holder fix in microtome, it arranged thickening of piece. The slicetion turn at hook microtom. It is in cross piece.
7. Affixing. The piece of preparat adhesived to glass obyec by albumine and gliserrine(1:1). Their were save in box for 1 day.
8. Deparaffinisasi. The deparaffination preparat entered liquid xylol for 10 minute.
9. Staining. The histology preparetion seed xylol by filter paper. Their entered to liquid alcohol 96%, 90%, 80%, 70%, 60%, 50%, 40 % and 30 % for 5 minute each, then entered to aquades for 5 minute, to hematoxyline dye for 7 second. After dying preparat entered to aquades and alcohol 50%, 60%, 70%, 80%, 90%, 96% for several dip. The immersion second is eosin for 5 minute. Then their entered to alcohol 70 %, 80 %, 90 %, 95 %. The preparats were dryed wind sirculating and their entered to xylol for 15 minute and their dropped canada balsem.
10. Mounting and Labelling. The mounting preparat was glas mount and it make title of preparat.

The histopatological observation of mice cancer cell was done by using a microscope with 400x magnification. The observation of praparates was done five times in different fields. Criteria of the cancer cell growth observation was based on (Table 1) [5]. Furthermore, data on histopatology were analyzed by using the Kruskall Wallis non-parametric statistic test.

III. Result and Discussion

Carcinogenic induction by injecting the Benzo(a)pyren of 0.3 gram/Oleum olivarum of 0.2 ml resulted in appearance of fibrosarcoma cancer in servix of the mice. Macroscopically, the fibrosarcoma cancer in the mice were nodules in head, back, and neck (Fig. 1(a)).

The histopatological observation of mice showed fibrosarcoma characters, i.e. fibroblast cell with anaplastic change, irregular cellular structure and shape, necrosis and mitosis, and proliferated cellular mass that resulted in the increased thickness of skin dermis layer. In fibrosarcoma, cell had big size and multicleated giant cell [16]. Such characteristics of fibrosarcoma was found in Group II, IV, V and VI, while group III showed the recovery of skin tissue and fibroblast cell to be normal. Normal fibroblast cell form was thin in shape with small nuclei and colagen fiber around it [21].

A. Effect of the Extract of Marine Sponge Aaptos Suberitoides on Cancer Cell Mitosis

Mitosis was found in Groups II, IV, V, and VI with number of 3-4 cells with 400x magnification (Fig. 4 and 5). Mitosis was one of the parameters used to determine the severity of cancer cell [5].

Number of cell mitosis in Groups II, IV, V and VI was the same. It indicates that in the treatment group administered by the sponge extract (Group IV, V, and VI), the treatment still shows the high level of cell mitosis [6], while in Group III and I, which were administered by cyclophospamide, there was no mitosis.

B. Effect of Marine Sponge Extracts on Nuclear Polymorphism

Nuclear polymorphisms were found in Group II, IV, V, and VI (Fig. 7 and 8). The shapes of nuclei were diverse or in irregular forms in all the cells and number of polymorphism was 100%. The irregular forms of nuclei was one of the characters of fibrosarcoma [16]. The nuclear polymorphism was also one of the parameters that can be used to determine the severity of cancer [5]. In Group I and III, the cells were oval in shape, small in size, and uniform in all the cells with many colagen fibers around them.

The percentage of nuclear polymorphism in Group IV, V, and VI were the same with Group II. The condition indicates that the treatment group of sponge extracts still showed the character of fibrosarcoma, i.e. irregular shapes of nuclei [6]. In Group I and III, there was no nuclear polymorphism.

C. Statistical Analysis using the Kruskall Wallis and Pair Comparison Tests

Result of the analysis by using the Kruskall Wallis test in histopathology showed that inhibition of cancer growth in the three treatment groups of sponge extracts with the concentration of 500 mg/kg, 1000 mg/kg, and

1500 mg/kg was not significanly different with that of the treatment group of CMC Na (Group II was not given any treatment). It means that the sponge extracts had no anticancer activities in inhibiting the fibrosarcoma. It occurred because in the three treatment groups of sponge extracts, damage in tissue and cell were the same with that in the CMC Na group with no treatment. The damages observed in tissue were necrosis, nuclear polymorphism, and mitosis figures found in the three groups with the treatment of sponge extracts was relatively the same with that found in the CMC Na Group.

The histological observation of mice showed fibrosarcoma character because Benzo(a)pyren is carcinogenic. Induction Benzo(a)pyren made cancer skin hypodermis layer of mice like nodul. It is manifestation of cell proliferation approximately. Benzo(a)pyrene is polycyclyc hidrocarbone prototypic that it is compound carcinogenic. It can make mutation gen p53, it is regulator gene in cycle cell. The mutation of gene p53 make cycle cell uncontrol result of cell proliferation approximately [19].

Benzo (a) Piren, compound polycyclic hydrocarbon aromatic is precarcinogenic strong coumpound. Enzymes cytocrome of P450 phase 1 in cel-cel liver make added group hydrocyl for molecule by epoxide intermediate. The groups epoxite were electrofil and reactive, used and broke synthesis DNA. Their can fault transcription DNA and product other protein [4]. Benzo(a)pyrene is compound carcinogenic make mutation gene is manifestation of broken chromosome or aberration.

Based on the analysis, it can be stated that sponge extracts was not effective in inhibiting the fibrosarcoma growth. It was because the sponge extracts used for the treatment was not a pure compound. The sponge extracts were ethanol extracts containing various organic compounds. They were still dominated by non-alcaloid organic compounds. Generally, a pure compound has a higher level of biological activities as anticancer agents provided during the purification process, the active compound were not dissapearred or severaly damaged [13]. The low content of alkaloid compound in sponge extracts was not able to repair tissue damages in the fibrosarcoma. It was due to the fact that the number of cancer cell which was destroyed by the treatment of sponge extract was less than new mass of cells resulted from the uncontrolled proliferation of cancer cells.

V. CONCLUSIONS

Based on results of the analysis, it can be concluded that the characters of fibrosarcoma were necrosis, mitotic figure, and nuclear polymorphism in Groups II, IV, V, and VI. Percentages of cell necrosis were 20-60%, mitotic figures were 3-4 cells with 400x magnification and percentage of nuclear polymorphism were 100%. In Groups I and II there was no cell necrosis, mitotic figure, and nuclear polymorphism. The etanol extracts of marine sponge *Aaptos suberitoides* had no anticancer activity in mice fibrosarcoma tissue.

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REFERENCES

[1] Aoki S., Dexin, K. Hideaki S., Yoshihiro, S., Toshiyuki, S., Andi ,S., Motomasa K., 2006, "Aaptamin, a Spongean Alkaloid, Activates p21 Promoter in a p53-Independent Manner", *Biochemical and Biophysical Research Communications*, Vol. 342, pp. 101-106.

[2] Astuti, P., Alam, G., Tahir, A., Wahyuono, S., 2002, "Toxicity studies of sponges collected from barrang lompo island against *artemia salina* leach", *Journal Traditional Medicine*, Vol. 7, No. 21, pp. 19-23.

[3] Astuti, P., 2005, "Uji sitotoksik senyawa alkaloid dari spons *petrosia* sp : potensial pengembangan sebagai antikanker", *Majalah Farmasi Indonesia*, Vol. 16, No.1, pp. 58-62.

[4] Ben Best. 2006. Cancer Death Causes & Prevention. Http://benzo/Hasil%20 Penelusuran%20Gambar%20Google%20-untuk%20 http//www_benbest_com-health-BenzoPyr_gif_files/cancer.htm. 3 Oktober 2009/13.15

[5] Couto.S.S, Grifey.S.M, Duarte.P.C, Madewell.B.R. 2002. "Feline Vaccine-Associated Fibrosarcoma : Morphological Distinctions" *Vet. Pathological* Vol.39, pp 33-41.

[6] Jimenez, Angel Garcia. Castellvi, Joseph. Frutoz, Isabella Diaz. 2009. "Clinocopathologic Consideration About The Intraoperative and Post Surgical Prosedure", *Case Report in Medicine.* Vol. 29, pp 1-4.

[7] Kumar.V, Abbas. A, Fausto.N, Mitchell. R. 2007. Robbins Basic Pathology. Saunders Elsevier. Philadelphia.

[8] Kurnijasanti, R.K, Hamid,I.S, Rahmawati,K. 2008. "Efek Sitotoksik In Vitro dari Ekstrak Buah Mahkota Dewa(*Phaleria macrocarpa*) Terhadap Kultur Sel Mieloma", *Jurnal Penelitian Medika Eksata*. Vol.7 No.1, pp 12-18.

[9] Meiyanto, E., and Septisetyani, E. P, 2005. "Efek Antiproliferatif dan Apoptosis Fraksi Fenolik Ekstraketanolik Daun Gynura Procumbens terhadap Sel HeLa" , *Jurnal Artocarpus*. Vol. *5*,No. 2, pp 74-80.

[10] Murray, Robert K, dkk. 2003. Biokimia Harper. Penerbit Buku Kedokteran EGC: Yakarta

[11] Nurhayati, Awik Puji. 2008. "Uji Aktivitas Antibakteri Dari Fraksi Methanol dan Klorofom Spons *Pseudosuberites andrewesi* Di Pantai Pasir Putih Situbondo Terhadap Bakteri *Staphylococcus aureus* ATCC 25923", *Penelitian Produktif DIPA ITS.*

[12] Nursaadah, Afifa. 2008. Uji Pendahuluan Ekstrak Spons *Spongosorites* sp. dari Pantai Pasir Putih Situbondo yang Berpotensi Sebagai Antibakteri. *Skripsi Program Studi Biologi ITS*

[13] Nursid, Muhammad. Wikanta, Thamrin. Fajarwati, Nurrahmi. 2006. "Aktivitas Sitotoksik, Induksi Apoptosis dan Ekspresi Gen P 53 Fraksi Metanol Ekstrak Spons Petrosia sp Terhadap Sel Tumor HeLa", *Jurnal Pascapanen dan Biotekonologi Kelautan dan Perikanan* Vol. 1 No 2, pp 103-110.

[14] Powers, Barbara and Dernell, William. 1998. "Tumor Biology and Patology", *Clinical Techniques in Small Animal Practice* Vol. 13, No.1, pp 97-102.

[15] Setyowati, Erna. 2005. Isolasi Senyawa Sitotoksik Spons Kaliapsis. *Majalah Farmasi Indonesia* Vol. 18, No.4, pp 183-189.

[16] Steele, Heather. 2001. "Subcutaneous Fibrosarcoma in an Age Guinea Pig", *Veterinary Journal*, Vol. 42, pp 300-302.

[17] Suryati dan. Ahmad 1996. "Spons Sumber Bakterisida Alami". *Trubus*. Edisi No. 18. Th. XXVII

[18] Van Soest, R.M.W. 1989. "The Indonesian Sponge Fauna": Status Report. *Netherland Journal of Sea Research*. Vol. 23 , No. 2, pp 223-230.

[19] Zakaria, Fransiska R., 2001., Pangan dan Pencegahan Kanker. *Jurnal Teknologi dan Industri Pangan*, Vol. 12, No. 2.
[20] Amir, I., and A Budiyanto, 1996, "Mengenal Spong Laut (Demospongiae) Secara Umum, Oseana Vol. 21, No. 2, pp. 15-31.
[21] Bavelander, G nad Goss,R.J. 1998. Influence of tetracycline on calcification in mormal and regerating teleost scales. Nature. 193, 1098-1099.

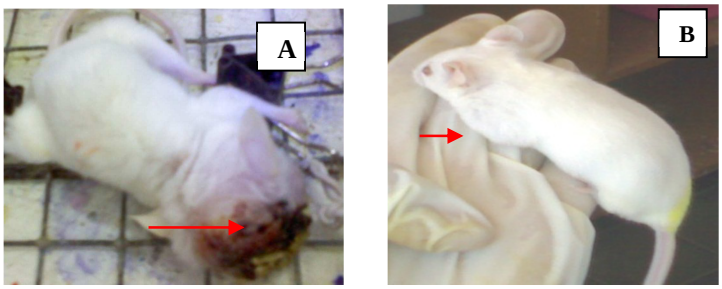

Fig. 1. Carcinogenic Induction by injecting the Benzo(a) pyren of 0.3/Oleum Olivarum of 0.2 ml in Cervix of the Mice: (A) Nodules In Head of the Mice with Cancer and (B) normal mice

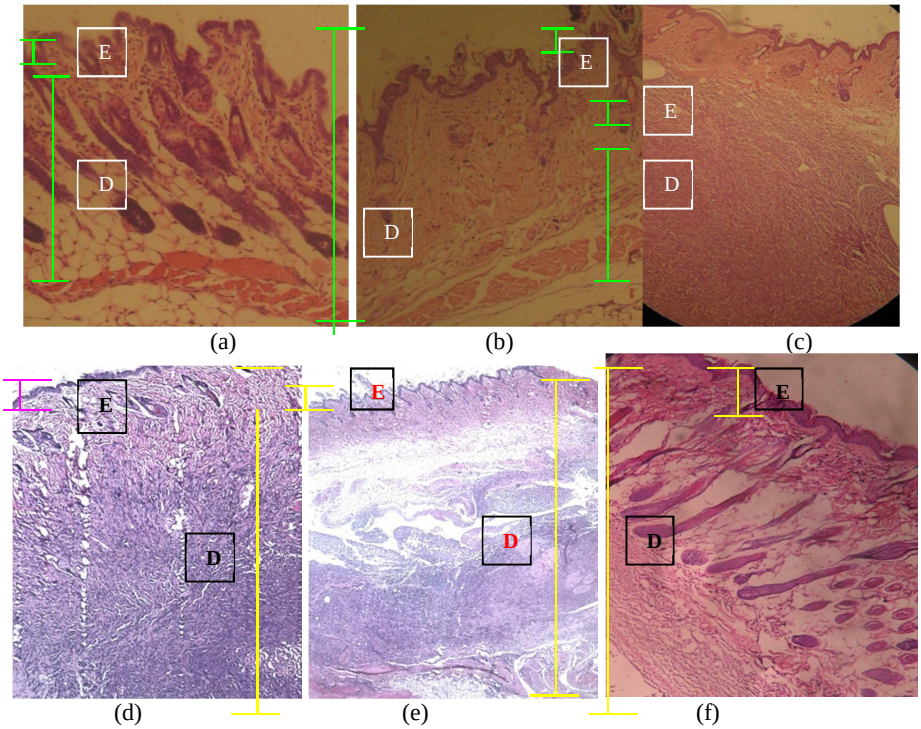

Fig. 2. Histological Section of Mice Skin: (a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V, (f) Group VI. Hematoxilin Eosin Staining 400x magnification E = Epidermis, D= Dermis

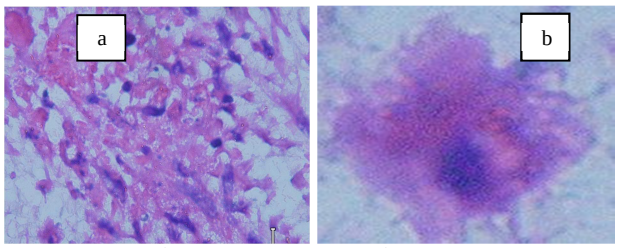

Fig. 3. Fibrosarcoma Tissue on In Vivo Evaluation of the Anticancer Extract of Marine Sponge *Aaptos Suberitoides*: (a) Necrotic Tissue, (b) Necrotic Cell

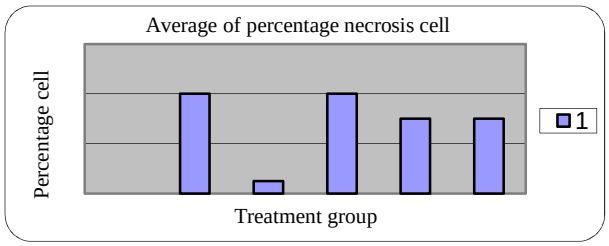

Fig. 4. Percentage of necrosis in mice skin tissue on in vivo evaluation of the anticancer extract of marine sponge *Aaptos Suberitoides*

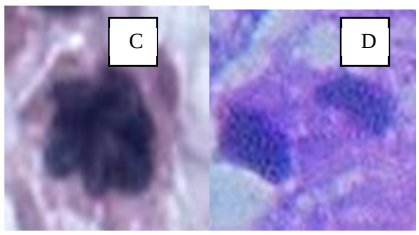

Fig. 5. Cell mitosis in *Fibrosarcoma Tissue*: (a) metaphase, (b) anaphase(c), prophase, and (d) telophase

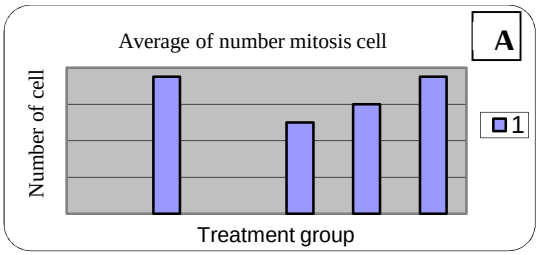

Fig. 6. Number of mitosis in each treatment

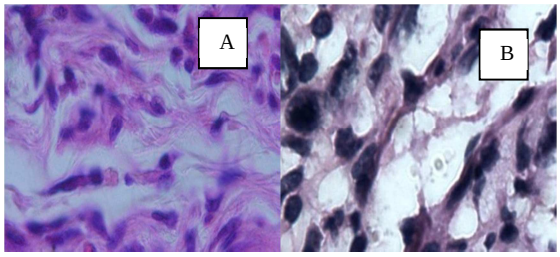

Fig. 7. Nuclear Polymorphism in *Fibrosarcoma Tissue*: (a) cell with normal nuclei, (b) cell with nulear polymorphism

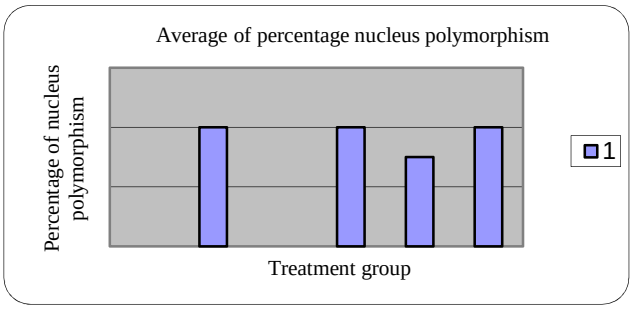

Fig. 8. The Percentage of nuclear polymorphism in all the treatments. repeated for 4

TABLE 1.
THE SCORES OF CANCER CELL GROWTH IN SUBCUTANEOUS SKIN TISSUE

Parameter	Score 1	Score 2	Score 3
Necrosis	No necrosis	necrosis < 50 % of the total area of the specimen	Necrosis > 50 % of the total area of the specimen.
Mitotic figure	1-9 mitotic figure per ten field with 400x magnification.	10-19 mitotic figure in ten with 400x magnification.	More than 20 mitotic figure in ten with 400x magnification.
Nuclear polymorphism	More than 75% of mature nuclei.	25 %- 75 % mature nuclei	- less than 25% of mature nuclei. - extremely irregular in nuclei form.