Research Journal of Biological Sciences 3 (12): 1437-1443, 2008
ISSN: 1815-8846

The Effect of a Natural Solution of Coconut Water on the Macrophage Cultures: A Morphological Analysis

[1]H.I.G. Bastos, [2]R.B. Freire, [2]H.R. Borba, [2]C.D. Coelho, [2]A.F. Rodrigues and [1,2]G.F. Diré
[1]Centro de Ciências da Saúde, Faculdade de Odontologia, Universidade Estácio de Sá,
Mestrado em Odontologia, Campus Barra World/Recreio, Av. Alfredo Baltazar da Silveira, 580,
Barra da Tijuca, Rio de Janeiro, RJ 22790701, Brazil
[2]Laboratório de Atividade Anti-helmíntica de Plantas, Departamento de Biologia Animal,
Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro,
Rio de Janeiro, R.J. 23890.000, Brazil

Abstract: Coconut (*Cocos nucifera* L.) is a monocotyledonous plant of the Arecaceae family. Coconut is a perennial tropical monocotyledon that produces fruit continuously. We aimed to determine the possible effects of coconut water on macrophage cells. Macrophages cells were obtained by Wistar rats and treated with coconut water solution during 30 and 60 min. Morphology microscope analysis has been done. Due to the analysis of the results it was observed that in the quantitative and in the qualitative analysis was observed the apoptotic effect of the coconut water in the cells culture treated during 30 and 60 min. It may be suggested that apoptotic effect of the referred studied solution would be related to the possible presence of a substance which may alter the imbalanced expression of iNOS/eNOS in the macrophage cells.

Key words: Macrophage, coconut water, nitric oxide, apoptosis, morphology

INTRODUCTION

During the accession and subsequent reimplant the bruise of the periodontal ligament with cellular necrosis occurs resulting in processes of repair of the wound where the necrotic periodontal ligament is removed by macrophages or cement for the osteoclastic activity. This last one will lead to the reabsorption of inflammatory surface or depending on the state of the pulp, of the age of the patient and the stage of development to the dental root. The inflammatory reabsorption can histological be demonstrated to one week after the reimplant and in the root it is more common in reimplanted immature teeth and young mature teeth that in older mature teeth. When great areas of the periodontal ligament are traumatized, the competitive cicatrisation of the wound starts enters the cells derived from the óssea marrow destined to form bone and cells derived from the periodontal ligament programmed to form staple fibres of this ligament and cement. The result of this competition can be an anchylosis of permanent transition. The absence of epithelial remaining portions of Malassez in the periodontal ligament dry by drawn out extra-alveolar periods can lead to the anchylosis occurrence because it is responsible for keeping the periodontal space (Andreasen, 1992; Lustosa-Pereira *et al*., 2006).

The necrosis is the type of the commonest cellular death, it wraps great cellular edema, denaturizing and coagulation of proteins, degradation of organelles cellular and break of cells, loss of the entirety of the plasmatic membranes, citoplasmatic disorganization and nuclear debauchery. In general, a great number of cells in the adjacent cloth is affected. The apoptosis takes place when the cell dies due to the activation of a program of suicide controlled internally, what wraps an orchestrated disturbance of cellular components; there takes place a least break of the adjacent cloth. Morphologically, it takes place to condensation and the fragmentation of the chromatin with degradation of the DNA genomic in fragments oligonucleisomas, loss of the volume and increase of the cellular granulocyte, maintenance of the structure of the organelles, formation of pleats in the plasmatic membrane and consequent cellular fragmentation in apoptotic bodies. In different cellular populations, a great similarity is observed in the phenotype of the apoptosis, same in quite different

Corresponding Author: Glaucio Diré Feliciano, Universidade Estácio de Sá, Faculdade de Odontologia, Mestrado em Odontologia, Centro de Ciências da Saúde, Campus Barra World/Recreio, Av. Alfredo Baltazar da Silveira, 580, Barra da Tijuca, Rio de Janeiro, RJ 22790701, Brazil

physiologic situations. This phenotype is resultant, principally, of the action in members waterfall of a special family of proteases cistein-aspartate. In different cellular populations, a great similarity is observed in the phenotype of the apoptosis, same in quite different physiologic situations. This phenotype is resultant, principally, of the action in members' waterfall of a special family of proteases cistein-aspartate, called Caspases. These are divided in 2 groups, being the executioners responsible for the execution of the process in you. Through the Caspases space, the fragments are produced oligonucleisomas characteristic of the apoptosis. The cells can die for apoptosis or mechanisms not-apoptotic proteases, called Caspases. These are divided in 2 groups, being the executioners responsible for the execution of the process in you. Through the Caspases space, the fragments are produced oligonucleisomas characteristic of the apoptosis. The cells can die for apoptosis or mechanisms not-apoptotic (Hortelano *et al*., 2001; Amarante-Mendes, 2003; Tabas, 2005; Mitchell *et al*., 2007).

Coconut (*Cocos nucifera L.*) is a monocotyledonous plant of the Arecaceae family. Coconut is a perennial tropical monocotyledon that produces fruit continuously. The physiological function of the large amounts of sucrose stored in coconut stems is unknown. Coconut water, which contains many uncharacterized phytohormones is extensively used as a growth promoting supplement in plant tissue culture. The omega-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA, 22:6 n-3) has been previously shown to facilitate some of the vital functions of astrocytes. Since, some dietary oils contain α-linolenic acid (ALA, 18:3 n-3), which is a precursor of DHA, we examined their effect on astrocyte development. Fatty acids (FAs) were isolated from commonly used oils and their compositions were determined by GLC. FAs from 3 oils, viz. coconut, mustard and linseed were studied for their effect on astrocyte morphology. Parallel studies were conducted with FAs from the same oils after heating for 72 h. Unlike coconut oil, FAs from mustard and linseed, both heated and raw, caused significant morphogenesis of astrocytes in culture. ss-AR binding was also substantially increased in astrocytes treated with FAs from raw mustard and linseed oils as compared to astrocytes grown in normal medium. The expression profile of the isoforms of GFAP showed that astrocyte maturation by FAs of mustard and linseed oil was associated with appearance of acidic variants of GFAP and disappearance of some neutral isoforms similar to that observed in cultures grown in serum containing medium or in the presence of DHA. Taken together, the study highlights the contribution of specific dietary oils in facilitating astrocyte development that can have potential impact on human health (Joardar and Das, 2007).

Dayrit *et al*. (2008) described that monoglycerides, diglycerides, sterols, free fatty acids and dioxaphospholane derivatives are the compounds present in the coconut water.

Koschek *et al*. (2007) investigated the *in vitro* anti-tumoral activities of fractions from aqueous extracts of the husk fiber of the typical A and common varieties of *Cocos nucifera* (Palmae). Due to the analysis of them it was observed that since, the common *C. nucifera* variety is extensively cultured in Brazil and the husk fiber is its industrial by-product, the results obtained in the their study suggested that it might be a very inexpensive source of new antineoplastic and anti-multidrug resistant drugs that warrants further investigation.

Sandhya and Rajamohan (2006) described that rats, who feeding coconut water resulted in increased plasma L-arginine content, urinary nitrite level and nitric oxide synthase activity and that these results indicate that both tender and mature coconut water has beneficial effects on serum and tissue lipid parameters in rats fed cholesterol-containing diet.

Vigliar *et al*. (2006) related that the biochemical profile of coconut water varied as the coconuts matured, observing reductions in the concentration of potassium, calcium, magnesium, chloride and osmolarity. Descending study chromatography revealed an increase in the concentration of fructose and glucose and also a reduction in the concentration of sucrose.

Gopikrishina *et al*. (2008) described that coconut water (CW) may be better alternative in terms of maintaining viable periodontal ligament cell viability after avulsion and storage.

Mantena *et al*. (2003) described that the scavenging ability and protection of hemoglobin from oxidation may be partly attributed to the ascorbic acid, which is an important constituent of CW. As CW is a rich source of vitamins, amino acids and enzymes etc., more than one active principle maybe involved.

In other study, Esquenazi *et al*. (2002) described that Water extract obtained from coconut husk fiber and fractions from adsorption chromatography revealed antimicrobial activity against *Staphylococcus aureus*. The crude extract and one of the fractions rich in catechin also showed inhibitory activity against acyclovir-resistant herpes simple x virus type 1 (HSV-1-ACVr). All fractions were inactive against the fungi *Candida albicans*, *Fonsecaea pedrosoi* and *Cryptococcus neoformans*. Catechin and epicatechin together with condensed tannins (B-type procyanidins) were demonstrated to be the components of the water extract.

The aim of this research was to evaluate the effect of the coconut water at cultures of macrophages obtained of Wistar rats.

MATERIALS AND METHODS

In the present research, macrophages were obtained of *Wistar* rats (*Rattus norvegicus albinus*). The animals (n = 5) used in the research was obtained of the enterprise Biocampo 2000 Biological Product Ltda (enterprise supplier of biological products, including animals for inquiries, done not not linked to the specific institution and what has an own bioterio in appropriate conditions of functioning). In the sequence for attainment of macrophages, first there was carried out the preparation of the way of culture where the cells would be put. A liter of distilled water was used. There happened, then, the preparation of the least essential way of Eagle whose pH is 7.0. A bottle of the powder of the way was poured in 1 L of distilled sterile water.

There was carried out positive filtration of the way Eagle through, sterile membrane of 0.22 μm. About 100 mg of penicillin were weighed G benzatine and 100 mg of sulphate of streptomycin. About 121 mg L^{-1} of penicillin and 230 mg L^{-1} of streptomycin (both the commercial mark Sigma-Aldrich, St Louis, USES) was obtained in the end. The antibiotics were put in the middle of culture, since east because of being a rich, even sterile way can become contaminated. Concentration of one was used mL of the antibiotic for each 50 mL of the way (concentration of 50 times). After getting the way of culture, the animal was put in a bottle containing cotton and chloroform that was closed. After the death of the animals, there was carried out the injection of the way of culture in the peritoneum of the same thing. The edema obtained through, this maneuver attracts macrophages. The macrophages were inhaled by syringe and transferred to tubes with way of culture. These tubes were stipulated in ice. Brackets of the content were transferred to a blade to observation of the cells. For the microscopic analysis, there was used the blade of Neubauer, which allows the placing of the material collected in a compartment of the blade to make easy the subsequent counting of the cells that in our case are the macrophages. The counting was carried out through a hemocitometry. To the end 25 mL was obtained of solution with macrophages. It was considered that 0.8×10^5 cells mL^{-1} for 1 mL, soon 200×10^5 is for 25 mL in proportion. It was obtained 2×10^7 cells of the rats in 5 min. These are migratory cells. In 100 μL have 1/10 of this value. Serum was added fetal bovine to 20%, which contains adesines what make easy the adhesion of the macrophages in the plate.

There were put brackets of the way of culture containing the macrophages in the wells of the plates for cultivation. The plates were put in a drier with environment of CO_2 (distilled water, bicarbonate of sodium (Na_2HCO_3) and sulphuric acid) creating an environment of anaerobiose. The drier was left in stove to 37°C. It was used 2 micro plates of test for cellular culture, with 6 orifices each one (Zellkultur Testplatte).

In a 2nd step, left for the exposure of macrophages to the means that are used in the avulsion dentistry. The means to be used preservatives were:

- The preservative coconut water.
- The positive control was the way with fetal bovine serum.
- The negative control was the medium containing Antimicina A (used as a lethal substance).

The preservative one was coconut water obtained from fresh coconut. For the negative control, was used to Antimicina A (Sigma-Aldrich, St. Louis, USA) that acts inhibiting the oxidation cell, inhibits cytochrome (complex I, NADH dehydrogenase) and therefore, there is no production of ATP in mitochondrial respiration of macrophages, causing cell death. A toxic dose of Antimicina A ($C_{20}H_{40}N_2O_4$) is 2 μg mL^{-1} and lethal dose is 200 DL50.

The funds were collected from plates. Two plates with 6 wells had their resources collected and put into test tubes and the preservatives were placed in the wells numbered 1-6, respectively in the plate with their numbers pre-established. These cards are numbered 1-3 at the top and from 4-6 at the bottom. One such plaque was placed for 30 and another for 60 min in dissector and left in the oven to 37°C. After the times of their 30 and 60 min, preservatives were collected and the plates quickly washed with PBS. The Blue of Trypan to 0.5%, was added to all wells and left for 1 min. The wells were washed with water and then added to methanol and then Panotic to fix the dye. After stained and fixed, the plates were observed under the microscope. Not much ruddy or clearer cells are those that show up cheer and the ruddiest or when they fled well, these one are the dead one.

RESULTS AND DISCUSSION

The macrophages were collect from Wistar rats and they were treated with different types of solutions. After a period of time the survive of these cells was analyzed and expressed by values in percentage.

The Table 1 has shown the comparison of the percentage of surviving between the control and treated groups in different times (30 and 60 min).

Table 1: Effect of coconut water in the survive of macrophages cells

Preservative ways	30 min (%)	60 min (%)
Positive control	100	100
Coconut water	17	34

Due to the analysis of the results it was observed that there was difference between the group treated with coconut water solution (30 and 60 min) to the control group ($p = 0.047$; Dunn's Multiple Comparison Test and Kruskal-Wallis test, by the Prism 5, statistical program analysis).

In the Fig. 1-4 (100 and 400x), could be observed that the extract of coconut water solution was capable of inducing to apoptosis in the different times of treatment (30 and 60 min) this difference was statistically observed.

Sim *et al*. (2008) described that coconut water was found to contain high level of zeatin riboside. Gopikrishna *et al*. (2008) related that coconut water is biologically pure and sterile, with a rich presence of amino acids, proteins, vitamins and minerals and that statistical analysis showed that coconut water kept significantly more periodontal ligament cells viable compared with propolis, Hank's balanced salt solution (HBSS), or milk as well as coconut water can be used as a superior transport medium for avulsed teeth. Although, in our experimental it was observed that coconut water induced a high expression of apoptosis in the macrophage cells. This fact would not indicate coconut water as a transport medium for avulsed theeth as described by Gopikrishna *et al*. (2008). Related to the inference due to the fact that coconut water is biologically pure and sterile we would speculated that coconut water is not once as described by Prabakaran *et al*. (2008) coconut water-based culture medium is economical for the production of *Bacillus thuringiensis* var. israelensis demonstrating the excellent biological condition of coconut water solution to the development of other types of contaminant microorganisms.

In other study Abara *et al*. (2007), described the hepatic protection effect of coconut water showed that the ingestion of coconut milk and coconut water increased the values of protein and protein/RNA ratios, it decreased alanine and aspartate amino transferase (ALT and AST) activities. These effects, in turn, enhanced the induction of the metabolizing enzymes and a resultant faster clearance and elimination of the caffeine from the body, there by reducing the toxic effect on the liver. Complementary, a study developed by Ismail *et al*. (2007) has showed that ingesting of coconut water was as good as ingesting a commercial sports drink for whole body rehydration after exercise-induced dehydration but with better fluid tolerance. It is known that Nitric oxide (NO) and Monocyte Chemoattractant Protein (MCP)-1

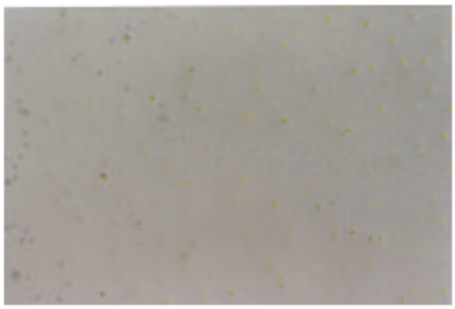

Fig. 1: Negative control. All the dead cells (100x)

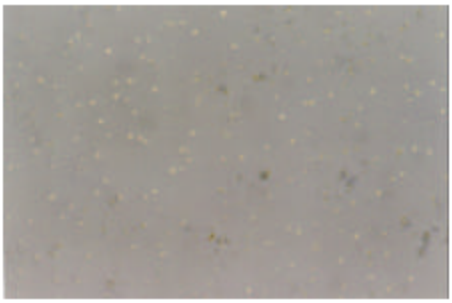

Fig. 2: Control positive. Virtually all living cells (100x)

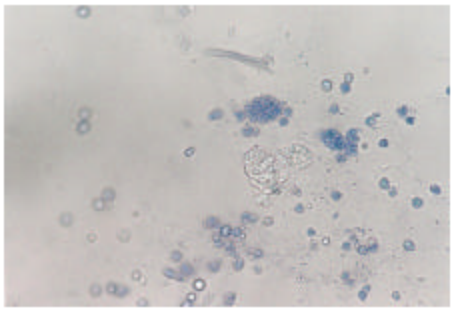

Fig. 3: Coconut water in 30 min. Cells killed, degenerated, the presence of bodies apoptolic (400x)

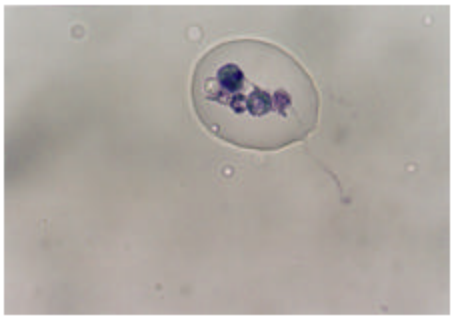

Fig. 4: Coconut water in 60 min Apoptosis (400x)

co-regulation has been found in endotoxin-activated macrophages. Kupffer cells (KC) are a main source of soluble-mediators production in liver abnormalities. Kolios *et al*. (2008) indicated that KC might be the main source of NO and MCP-1 production in liver disorders, probably through, the induction of PI3-kinase (s) and without any co-regulation between these molecules, which might represent two independent immun-oregulatory pathways in the role of KC in hepatic disorders. Due to the analysis of the results found in this research, may be the apoptosis level observed would be related to the increased of depuration activity which would be related to the macrophage in the liver as the Kupffer cells this hypothesis would be supported by the study developed by Sandhya and Rajamahan (2006), whom described that an increased rate of cholesterol conversion to bile acid and an increased excretion of bile acids and neutral sterols were observed in rats fed coconut water and that in the histopathological studies of liver and aorta revealed much less fatty accumulation in these tissues in cholesterol-fed rats supplemented with coconut water. They observed that feeding coconut water resulted in increased plasma L-arginine content, urinary nitrite level and nitric oxide synthase activity. These results indicated that both tender and mature coconut water has beneficial effects on serum and tissue lipid parameters in rats fed cholesterol-containing diet.

Transgenic overexpression of calcineurin (CN/Tg) in mouse cardiac myocytes results in hypertrophy followed by dilation, dysfunction and sudden death. It was described that Nitric oxide produced via inducible nitric oxide synthase (iNOS) has been implicated in cardiac injury. Since, calcineurin regulates iNOS expression and since, phenotypes of mice overexpressing iNOS are similar to CN/Tg, we hypothesized that iNOS is pathogenically involved in cardiac phenotypes of CN/Tg mice. Calcineurin activates local production of NO by iNOS in cardiac myocytes which significantly contributes to sudden death, heart block, LV dilation and impaired systolic performance in this murine, model of cardiac hypertrophy induced by overexpression of calcineurin (Somers *et al*., 2008). This fact would be related to the apoptotic effect induced coconut water in the macrophages once as described by other authors coconut water induced the increased of protein and protein/RNA ratios as well as the level of nitric oxide synthase activity together with the L-arginine level, maybe this potential mechanisms would be the responsible by the apoptotic effect in the macrophages isolated cells in our *in vitro* study. Inflammation and oxidant stress have been suggested to be involved in the structural remodeling in atrial fibrillation (AF) and inducible nitric oxide synthase (iNOS) is associated with inflammation and oxidant stress. Han *et al*. (2008) described that there is a novel evidence that imbalanced expression of iNOS/eNOS which could contribute to protein nitration and cardiomyocyte apoptosis in human in which condition inflammation may be an important participant.

According to Busshan *et al*. (2007), triterpenediol (TPD) comprising of isomeric mixture of 3α, 24-dihydroxyurs- 12-β and 3α, 24-dihydroxyolean- 12-β from *Boswellia serrata* induces apoptosis in cancer cells. These studies thus demonstrate that TPD produces oxidative stress in cancer cells that triggers self-demise by reactive oxygen species (ROS) and NO regulated activation of both the intrinsic and extrinsic signaling cascades. Nakamura *et al*. (2008) screened the inhibitory effect of the extract from 50 Thai medicinal plants on an inducible-nitric oxide synthase (iNOS) expression induced by lipopoly-saccharide (LPS) in mouse macrophages RAW 264.7. From this screening, the extracts of root bark of *Clausena guillauminii*, *C. lunulata* and *C. excavata* (*Rutaceae*) were found as the extracts which showed potent inhibitory effect on the iNOS protein expression in concentration-dependent manner. Furthermore, in fact we may suggest that a similar effect would be related with the effect of coconut water in the apoptotic mechanism observed in this study.

CONCLUSION

In the light of the results we may speculate that the apoptotic effect related to the coconut water would be related to the possible presence of a substance which may alter the imbalanced expression of iNOS/eNOS in the macrophage cells.

REFERENCES

Abara, A.E., G.O. Obochi, M. Obi-Abang, S.P. Malu, V.S. Ekam, F.E. Uboh and F.E. Edu, 2007. Effect of caffeine-coconut products interactions on induction of microsomal drug-metabolizing enzymes in Wistar Albino rats. Nig. J. Physiol. Sci., 22 (1-2): 75-81. DOI: 10.1126/science 286.5443.1347. PMID: 18379623. http://www.bioline.org.br/abstract?id=np07013&lang=en.

Amarante-Mendes, G.P., 2003. Apoptose: Programa molecular de morte cellular. Einstein, 1: 15-18. http://www.einstein.br/biblioteca/pdf/On%20line%2010.pdf.

Andreasen, J.O., 1992. Atlas of Replantation and Transplantation of Teeth. Switzerland: Medi Globe. ISBN: 0721667112 9780721667119 2882390157 9782882390158.

Bhushan, S., A. Kumar, F. Malik, S.S. Andotra, V.K. Sethi, I.P. Kaur, S.C. Taneja, G.N. Qazi and J.A. Singh, 2007. Triterpenediol from *Boswellia serrata* induces apoptosis through, both the intrinsic and extrinsic apoptotic pathways in human leukemia HL-60 cells. Apoptosis, 12 (10): 1911-26. DOI: 10.1007/s10495-007-0105-5. PMID: 17636381. http://www.springerlink.com/content/v81t71h625p26t03/.

Dayrit, F.M., O.E. Buenafe, E.T. Chainani and I.M. de Vera, 2008. Analysis of monoglycerides, diglycerides, sterols and free fatty acids in coconut (*Cocos nucifera* L.) oil by 31P NMR spectroscopy. J. Agric. Food Chem., 56 (14): 5765-5769. PMID: 18576656.

Esquenazi, D., M.D. Wigg, M.M. Miranda, H.M. Rodrigues, J.B. Tostes, S. Rozental, A.J. da Silva and C.S. Alviano, 2002. Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res. Microbiol., 153 (10): 647-652. DOI: 10.1016/S0923-2508(02)01377-3. PMID: 12558183. http://grande.nal.usda.gov/ibids/index.php?mode2=detail&origin=ibids_references&therow=729690.

Gopikrishna, V., P.S. Baweja, N. Venkateshbabu, T. Thomas and D. Kandaswamy, 2008. Comparison of coconut water, propolis, HBSS and milk on PDL cell survival. J. Endod., 34 (5): 587-589. PMID: 18436040.

Gopikrishna, V., T. Thomas and D.A. Kandaswamy, 2008. quantitative analysis of coconut water: A new storage media for avulsed teeth. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod., 105 (2): 61-65. PMID: 18230380.

Han, W., S. Fu, N. Wei, B. Xie, W. Li, S. Yang, Y. Li, Z. Liang and H. Huo, 2008. Nitric oxide over-production derived from inducible nitric oxide synthase increases cardiomyocyte apoptosis in human atrial fibrillation. International Journal of Cardiology (Epub ahead of print). PMID: 18585800.

Hortelano, S., M.L. García-Martín, S. Cerdán, A. Castrillo, A.M. Alvarez and L. Boscá, 2001. Intracellular water motion decreases in apoptotic macrophages after caspase activation. Cell Death and Differentiation, pp: 1022-1028. PMID: 11598800.

Ismail, I., R. Singh and R.G. Sirisinghe, 2007. Rehydration with sodium-enriched coconut water after exercise-induced dehydration. Southeast Asian J. Trop. Med. Public. Health, 38 (4): 769-85. PMID: 17883020.

Joardar, A. and S. Das, 2007. Effect of fatty acids isolated from edible oils like mustard, linseed o r coconut on astrocytes maturation. Cell. Mol. Neurobiol., 27 (8): 973-83. DOI: 10.1007/s10571-007-9204-7. PMID: 17823864. http://www.springerlink.com/content/343r167t7q71m563/.

Kolios, G., V. Valatas, P. Manousou, C. Xidakis, G. Notas and E. Kouroumalis, 2008. Nitric oxide and MCP-1 regulation in LPS activated rat Kupffer cells. Molecular Cell. Biochemistry (Epub ahead of print). PMID: 18629611.

Koschek, P.R., D.S. Alviano, C.S. Alviano and C.R. Gattass, 2007. The husk fiber of *Cocos nucifera* L. (Palmae) is a source of anti-neoplastic activity. Braz. J. Med. Biol. Res., 40 (10): 1339-1343. PMID: 17713650.

Lustosa-Pereira, A., R.B. Garcia, I.G. de Moraes, N. Bernardineli, C.M. Bramante and E.A. Bortoluzzi, 2006. Evaluation of the topical effect of alendronate on the root surface of extracted and replanted teeth. Microscopic analysison rats' teeth. Endod. Dent. Traumatol., 22: 30-35. DOI: 10.1111/j.1600-9657.2006.00417.x. PMID: 16422756. http://www.ingentaconnect.com/content/mksg/edt/2006/00000022/00000001/art00006.

Mantena, S.K., Jagadish, S.R. Badduri, K.B. Siripurapu and M.K. Unnikrishnan, 2003. *In vitro* evaluation of antioxidant properties of *Cocos nucifera* Linn. Water. Nahrung, 47 (2): 126-31. PMID: 12744292.

Mitchell, R.N., V. Kumar, A.K. Abbas and N. Fausto, 2007. 8th Edn. W.B. Saunders, Philadelphia. ISBN: 853521836X.

Nakamura, T., N. Kodama, T. Kumamoto, Y. Higuchi, C. Chaichantipyuth, K. Ueno, T. Ishikawa and S. Yano, 2008. Inhibitory effect of the extracts from Thai medicinal plants on iNOS expression in mouse macrophage RAW 264.7. Nat. Med. (Tokyo) Vol: 15. PMID: 18626726.

Prabakaran, G., S.L. Hoti, A.M. Manonmani and K. Balaraman, 2008. Coconut water as a cheap source for the production of delta endotoxin of *Bacillus thuringiensis* var. israelensis, a mosquito control agent. Acta Trop., 105 (1): 35-58.

Sandhya, V.G. and T. Rajamohan, 2006. Beneficial effects of coconut water feeding on lipid metabolism in cholesterol-fed rats. J. Med. Food, 9 (3): 400-407. DOI: 10.1089/jmf.2006.9.400. PMID: 17004906. http://www.liebertonline.com/doi/abs/10.1089/jmf.2006.9.400.

Sim, G.E., C.J. Goh and C.S. Loh, 2008. Induction of *in vitro* flowering in Dendrobium Madame Thong-In (Orchidaceae) seedlings is associated with increase in endogenous N (6)- (Delta (2)-isopentenyl)-adenine (iP) and N (6)-(Delta (2)-isopentenyl)-adenosine (iPA) levels. Plant Cell. Rep., 27 (8): 1281-1289.

Somers, J.R., P.L. Beck, J.P. Lees-Miller, D. Roach, Y. Li, S. Loken, S. Zhan, L. Semeniuk, H.J. Duff, 2008. iNOS in Cardiac Myocytes Plays a Critical Role in Death in a Murine Model of Hypertrophy Induced by Calcineurin. American Journal of Physiology Heart Circ. Physiol. (Epub ahead of print). DOI: 10.1152/ajpheart.00386.2008. PMID: 18621856. http://ajpheart.physiology.org/cgi/content/abstract/00386.2008v1.

Tabas, I., 2005. Conseqüences and therapeutic implications of macrophage apoptosis in atherosclerosis. The importance of lesion stage and phagocytic efficiency. Arteriosclerosis, Thrombosis and Vascular Biol., 25: 2255-2264. PMID: 16141399. http://cat.inist.fr/?aModele=afficheN&cpsidt=17248026.

Vigliar, R., V.L. Sdepanian and U. Fagundes-Neto, 2006. Biochemical profile of coconut water from coconut palms planted in an inland region. J. Pediatr., 82 (4): 308-12. DOI: 10.2223/JPED.1508. PMID: 16912834. http://www.scielo.br/pdf/jped/v82n4/v82n4a14.pdf.