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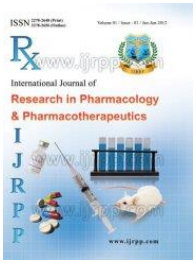

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Evaluation of anti-hemolytic activity of green tea aqueous extracts by *in vitro* method

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ABSTRACT
Hemolysis is a disorder of destruction of RBC cells in our body; chloroquine is the drug that regularly preferred to anti-malarial activity. This current study observes the destruction of RBC cells by chloroquine when treated with malarial disease and control of RBC lysis with addition of green tea extract along with chloroquine. Green tea leaves consist of phenolic compounds like flavonoids and catechin. Due to the presence of flavonoids and catechin which reduce the hemolysis of RBC by oxidative stress. This current study deals with concentration of hemolysis when induced with chloroquine and compared with aqueous extract of green tea with induced chloroquine and RBC suspension. This was clearly done by using the separation of the serum from plasma and induced the chloroquine to RBC suspension then the Heme generated was assayed and also absorbance was seen in UV visible photometry by following concentrations [high (2mg/ml) and low (1mg/ml)] of chloroquine. This same was repeated with addition of aqueous extraction of green tea by same concentration [high (1mg/ml) and low (2mg/ml)]. And observed that hemolysis taken place was 38.27% to 15.3%. (at low concentrations of chloroquine and green tea extract (1 mg/ml)). And also 55.56% to 26.17% (at high concentrations of chloroquine and green tea extract (2mg/ml)).

Keywords: Hemolysis, Chloroquine, Green tea leaves, Oxidative stress

INTRODUCTION

Hemolysis is classically understood as the release of hemoglobin and other intracellular components from erythrocytes to the surrounding plasma, following damage or disruption of the cell membrane. Hemolysis may occur either in vivo or in vitro, and is a most undesirable condition that influences the accuracy and reliability of laboratory testing [1]. Along with pre-analytical causes, in vivo blood cell lysis can originate from hereditary, acquired, and iatrogenic conditions, such as autoimmune hemolytic anemia, severe infections,

intravascular disseminated coagulation and transfusion reactions; it does not depend on the technique of the healthcare provider and is thus virtually unavoidable and cannot be resolved [2]. Visible hemolysis, as a hallmark of a more generalized process of blood cell damage, is usually not apparent until the separation of serum or plasma has occurred. It is commonly defined as an extracellular hemoglobin concentration of >0.3 g/L (4.65 mol/L), resulting in a detectable pink-to-red hue of serum or plasma with a visible appearance in specimens containing as low as 0.5% hemolysate [3].

Medicinal plants are the rich source of medicinally important compounds and since ancient time, plants and plant derived products are used as medicine in traditional and folk medicinal system. Initially the herbal drugs were used in the form of dried powder, gums, extracts or formulations of more than one plant products. Advanced scientific techniques brought a revaluation in herbal medicine industry and all focus is concentrate on active principles (bioactive molecule). However, a lot of processing is required to develop a drug from the natural sources. Toxicity of the active molecule is a key factor during drug designing, and hemolytic activity represents a useful starting point in this regard, it provides the primary information on the interaction between molecules and biological entities at cellular level. Hemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells (Da Silva et al., 2004) [4]. In vitro hemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on the human erythrocytes.

Malaria is an endemic infectious disease causing morbidity and mortality in tropical and subtropical areas of the world. Most deaths from malaria occur among children living in Africa where a child dies every minute. Malaria is caused by Plasmodium parasites which are spread to people through the bites of infected Anopheles mosquitoes. Although an effective vaccine is the best long term control for malaria, it is still not available. The global strategy for malaria control mainly focused on treatment using antimalarials to reduce or eliminate parasites. However, the emerging of drug resistant malaria parasites and insecticide resistant Anopheles mosquitoes has limited adequate treatment of malaria [4,5,6].Therefore, there is an urgent need develop new antimalarials to fight with the parasites One way is to isolate new antimalarial compounds from plants that are not yet fully explored.

The effective antimalarial activity of the two plant based drugs, quinine and artemisinin [7], has generated much interest to explore other plant resources for their possible antimalarial efficacy. Tea (Camellia sinensis), originated in China, is a widely-consumed beverage throughout the world. The growing interest in the health benefit of tea has prompted numerous investigations on their biological properties. There are two major kinds of tea, black and green tea. Both of them contain large amounts of phenolic substances consisting of catechin in green tea and the a flavin in black tea [8].It was suggested that activities of tea polyphenol are mostly due to their powerful scavenging and antioxidant activity [9].

Antioxidant tea components are reported to have beneficial protective effects against cancers and pathogenic microorganisms [10,11].In addition to antioxidant, green and black tea extracts have been shown to improve erythrocyte survival in vivo during oxidative stress condition [12,13].Moreover, green and black tea extracts have been reported to be more effective against oxidative stress-induced erythrocyte hemolysis [14].It has been suggested that oxidative stress is able to induce hemolysis by increasing of permeability of erythrocyte membrane, and polyphenolic content especially catechins and theaflavins have protective effects of hemolysis by maintain and reduce oxidative stress condition. Furthermore, correlation between Plasmodium parasite growth or parasitemia and anemia has been studied. Severe malarial anemia is a major complication of malaria infection and is multi-factorial resulting from loss of circulating erythrocytes from parasite replication, as well as immune mediated mechanisms [15, 16, 17]. Recently, antimalarial activity of green tea in both crude extract as well as some its major polyphenolic content has been observed in P. falciparum in vitro [18,19].

The major phenolic content in green tea is catechins, and it has been previously demonstrated the antimalarial activity. It was found that antioxidant activity of green tea catechins correlate to antimalarial property, especially the interference with

fatty acid biosynthesis may represent a primary mechanism to explain the observed in vitro growth inhibition effects [20]. In the present study, we aim to evaluate the antihemolytic activity of Green Tea.

MATERIALS AND METHODS

The following materials and methods are used in the present study

Materials Chemicals

- Alcohol 95%
- Chloroquine
- Triton X-100
- Ethylenediaminetetraacetic acid (EDTA)
- Sodium Chloride

Apparatus and Instruments

- Microcentrifuge tubes
- Microcentrifuge
- Micropipettes
- *UV Spectrophotometer*

Plant Materials

Green Tea collected from local market.

Preparation of Green Tea Extract (10mg/ml)

Dried leaves of green tea (*Camellia sinensis* L.) were purchased from a Local Market s For extraction, 1 g of ground leaves of each tea sample was extracted with 100 ml of distilled water (DW) at constant temperature of 95 °C under continuous Stirring .The supernatant was Subsequently filtered through Whatman No. 1 filter paper to remove rough particles and then centrifuged at 3,000 rpm for 10 min. The supernatant, called green tea crude extracts (GTE) was stored at 2–4 °C until analyzed.

Phytochemical studies for the Green Tea Extract

We done the phytochemical analysis and we observed that presence of flavonoids in the Green Tea Extract.

Phytochemical analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was performed by the extracts.

Test for Steroids and Triterpenoids

Liebermann Burchard test

Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Test for Glycosides

Keller Killiani Test

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Bromine water test

Test solution was dissolved in bromine water and observed for the formation of yellow precipitate to show a positive result for the presence of glycosides.

Test for Saponins

Foam Test

Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Alkaloids

Hager's Test

Test solution was treated with few drops of Hager's reagent (saturated picric acid solution).Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Flavonoids

Ferric chloride test

Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test

Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow

color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test

Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

Red blood cell suspension

Blood was obtained by venipuncture from healthy male volunteers (18 to 22 years old) collected in presence of anticoagulant tubes and centrifuged at 3,500 rpm for 15 min. Plasma and Buffy coat were removed. Red blood cells (RBCs) were suspended in 10 volumes of 0.9% NaCl and centrifuged at 2,500 rpm for 5 min. The RBCs were washed three times with the same solution. During the last washing, the packed cells were re-suspended in 10 volumes of phosphate buffered saline (PBS, pH 7.4) and utilized for the following assay.

Negative Control

Absorbance corresponding to 0% hemolysis was determined by adding 100µl of PBS to 50µl of erythrocyte suspension. To determine the absorbance we made to 1ml solution by adding 850µl of PBS.

Positive Control

Absorbance corresponding to 100% hemolysis was determined by adding 100µl of Triton X-100 to 50µl of erythrocyte suspension. To determine the absorbance we made to 1ml solution by adding 850µl of PBS.

Hemolysis Assay [59]

The Hemolysis assay was done as described by Henkelman.S. et al 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 40°C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with Normal saline and PBS, pH 7.4 for 5 min. Washed erythrocytes were stored at 4°C and used within 6 h for the hemolysis assay. 50 µl of 10 dilution (100 µl Erythrocytes suspension: 900 µl PBS) of erythrocytes suspension was mixed with 100 µl of test samples (1 and 2mg/ml), 100 µl of PBS was used as negative control and 100 µl of 1% Triton-X as positive controls. Reaction mixture was incubated at 370C water bath for 60 min. The volume of reaction mixture was made up to 1 ml by adding 850 µl of PBs. Finally it was centrifuged at 3000rpm for 3min and the resulting hemoglobin in supernatant was measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin.

The treatment groups are followed as

- Group 1-Negative control received only PBS plus RBC suspension
- Group 2-Positive control received Triton X-100 1%v/v plus RBC suspension
- Group 3-Chloroquine low concentration (1mg/ml) plus RBC suspension
- Group 4-Chloroquine high concentration (2 mg/ml) plus RBC suspension
- Group 5-Chloroquine low concentration plus RBC Suspension plus GTE (1mg/ml)
- Group 6 - Chloroquine high concentration plus RBC Suspension plus GTE (2mg/ml).

Calculation

The following formula was used to find out the hemolytic activity.

$$\% \text{ Hemolysis} = \frac{A_{sample} - A_{buffer}}{A_{max} - A_{buffer}} \times 100$$

Where ‘A sample’ is A540 of red blood cells with sample solution in PBS, ‘A buffer’ is A540 of red blood cells in PBS, and ‘A max’ is A540 of red blood cells with 1% (v/v) Tri-ton X-100 in PBS. No hemolysis (0%) and full hemolysis (100%) were observed in the presence of PBS and 1% (v/v) Triton X-100, respectively [60].

RESULTS

The performed uv results are stated in the table: 01.

Tables and figures

% Hemolysis obtained results by UV spectrophotometry

Table 01

S.No.	Groups	Absorbance	%Hemolysis
1	NC	0.002	0
2	PC	0.812	100
3	CHL(low)+RBCS	0.312	38.27
4	CHL(High)+RBCS	0.452	55.56
5	CHL(low)+RBCS+GTE(low)	0.126	15.3
6	CHL(High)+RBCS+GTE(high)	0.214	26.17

NC = Normal Control
PC = Positive Control
CHL=Chloroquine
RBCS= Red Blood Cell Suspension
GTE= Green Tea Extract

DISCUSSION

The directly proportional concentration of green tea extract along with choloroquine showed the better results and finally and was redued to the 26% of hemolysis at high concentrations and 15.6 at the low concentrations from 55% and 38% repectively (uv analysis before the addition of the green the extract). The percentage reduced with the addition of the green tea extract was found to be 47.10% and 39.47% respectively. And We have demonstrated anti-hemolytic effects of aqueous extracts from green tea, that contain polyphenol, in the maintenance of hematocrit in normal level in combination with standard antimalarials. Without antagonistic effect, it is suggested that tea extracts could be used as supplements to prevent anemia during treatment of malaria infection with standard antimalarial drugs. The investigation into the biologically active compounds containing in the aqueous extracts of green and black tea that exert effects on red blood cells as well as to understand the mechanism of hemolytic protection during *Plasmodium* infections being explored. The study would lead to the discovery of alternative ways that helps better treatment of malaria infection and reduce development of drug resistance by the parasite that is one of the major current problems for control of malaria.

CONCLUSION

We have demonstrated anti-hemolytic effects of aqueous extracts from green tea that contain polyphenol, in the maintenance of hematocrit in normal level in combination with standard antimalarials. Without antagonistic effect, it is suggested that tea extracts could be used as supplements to prevent anemia during treatment of malaria infection with standard antimalarial drugs. The investigation into the biologically active compounds containing in the aqueous extracts of green and black tea that exert effects on red blood cells as well as to understand the mechanism of hemolytic protection during *Plasmodium* infections being explored. The study would lead to the discovery of alternative ways that helps better treatment of malaria infection and reduce development of drug resistance by the parasite that is one of the major current problems for control of malaria.

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REFERENCES

[1]. Carraro P, Servidio G, Plebani M. Hemolyzed specimens: a reason for rejection or a clinical challenge? Clin Chem 46, 2000, 306–7.

[2]. Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. J Am Med Assoc 293, 2005, 1653–62.

[3]. Burns ER, Yoshikawa N. Hemolysis in serum samples drawn by emergency department personnel versus laboratory phlebotomists. Lab Med 33, 2002, 378–80.

[4]. Da Silva E, Shahgaldian P, Coleman AW, Haemolytic properties of some water-soluble para-sulphonato-calix-[n]-arenes. Int J Pharm. 273(1-2), 2004, 57-62.

[5]. WHO, MMWR Morb. Mortal Wkly. Rep. 59, 2010, 217.

[6]. S. Mharakurwa, T. Kumwenda, M. A. Mkulama, M. Musapa, S. Chishimba, C. J. Shiff, D. J. Sullivan, P. E. Thuma, K. Liu, and P. Agre, Proc. Natl. Acad. Sci. USA 108, 2011, 18796.

[7]. L. L. Koekemoer, B. L. Spillings, R. N. Christian, T. C. Lo, M. L. Kaiser, R. A. Norton, S. V. Oliver, K. S. Choi, B. D. Brooke, R. H. Hunt, and M. Coetzee, Vector Borne Zoonotic Dis. 11, 2011, 1193.

[8]. F. Astelbauer, M. Gruber, B. Brem, H. Greger, A. Obwaller, G. Wernsdorfer, K. Congpuong, W. H. Wernsdorfer, and J. Walochnik, Acta Trop. 123, 2012, 96.

[9]. S. Sang, J. D. Lambert, C. T. Ho, and C. S. Yang, Pharmacol. Res. 64, 2011, 87.

[10]. K. S. Almurshed, Saudi Med. J. 27, 2006, 1804.

[11]. G. Mikutis, H. Karakose, R. Jaiswal, A. Legresley, T. Islam, M. Fernandez-Lahore, and N. Kuhnert, Food Funct. 2012.

[12]. N. J. Naderi, M. Niakan, M. J. K. Fard, and S. Zardi, J. Dent. (Tehran) 8, 2011, 55.

[13]. V. B. Schini-Kerth, N. Etienne-Selloum, T. Chataigneau, and C. Auger, Planta Med. 77, 2011, 1161.

[14]. J. Halder and A. N. Bhaduri, Biochem. Biophys. Res. Commun. 244, 1998, 903.

[15]. E. Satoh, N. Tohyama, and M. Nishimura, Int. J. Food Sci. Nutr. 56, 2005, 551.

[16]. T. J. Lamb and J. Langhorne, Malar. J. 7, 2008, 68.

[17]. S. M. Ahmed, S. K. Abd Al-Rhim, A. A. Mohamedani, A. B. Habour, and A. A. Sadek, Saudi Med. J. 23, 2002, 684.

[18]. H. U. Okafor and O. Nwaiwu, J. Trop. Pediatr. 47, 2001, 271.

[19]. S. K. Sharma, P. Parasuraman, G. Kumar, N. Surolia, and A. Surolia, J. Med. Chem. 50, 2007, 765.

[20]. A. R. Sannella, L. Messori, A. Casini, F. F. Vincieri, A. R. Bilia, G. Majori, and C. Severini, Biochem. Biophys. Res. Commun. 353, 2007, 177 Chadburn A. The spleen: anatomy and anatomical function. Semin Hematol 37(1), 2000, 13-21.

[21]. Walters MC, Abelson HT. Interpretation of the complete blood count. Pediatr Clin North Am 43, 1996, 599-622.

[22]. Marchand A, Galen S, Van Lente F. The predictive value of serum haptoglobin in hemolytic disease. JAMA 243, 1980, 1909-11.

[23]. Tabbara IA. Hemolytic anemias. Diagnosis and management. Med Clin North Am 76, 1992, 649-68.

[24]. Gutman JD, Kotton CN, Kratz A. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 29-2003. A 60-year-old man with fever, rigors, and sweats. N Engl J Med 349, 2003, 1168-75.

[25]. Ucar K. Clinical presentation and management of hemolytic anemias. Oncology [Huntingt] 16(9-10), 2002, 163-70.

[26]. Maedel L, Sommer S. Morphologic changes in erythrocytes. Vol. 4. Chicago: American Society for Clinical Pathology Press, 50, 52,66, 1993.

[27]. Engelfriet CP, Overbeeke MA, von dem Borne AE. Autoimmune hemolytic anemia. Semin Hematol 29, 1992, 3-12.

[28]. Jefferies LC. Transfusion therapy in autoimmune hemolytic anemia. Hematol Oncol Clin North Am 8, 1994, 1087-104.

[29]. Schwartz RS, Berkman EM, Silberstein LE. Autoimmune hemolytic anemias. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, et al., eds. Hematology: basic principles and practice. Philadelphia: Churchill Livingstone, 3, 2000, 624.
[30]. Saif MW. HIV-associated autoimmune hemolytic anemia: an update. AIDS Patient Care STDS 15, 2001, 217-24.
[31]. Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. Am J Hematol 69, 2002, 258-71.
[32]. Petz LD. Drug-induced autoimmune hemolytic anemia. Transfus Med Rev 7, 1993, 242-54.
[33]. Perrotta PL, Snyder EL. Non-infectious complications of transfusion therapy. Blood Rev 2001, 15:69-83.
[34]. Schrier SL. Extrinsic nonimmune hemolytic anemias. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, et al., eds. Hematology: basic principles and practice. 3d ed. Philadelphia: Churchill Livingstone, 2000, 630-8.
[35]. Berkowitz FE. Hemolysis and infection: categories and mechanisms of their interrelationship. Rev Infect Dis 13, 1991, 1151-62.
[36]. Barrett-Connor E. Anemia and infection. Am J Med 52, 1972, 242- 53.
[37]. Beutler E, Luzzatto L. Hemolytic anemia. Semin Hematol 36(4-7), 1999, 38-47.
[38]. Beutler E. G6PD deficiency. Blood 84, 1994, 3613-36.
[39]. Steensma DP, Hoyer JD, Fairbanks VF. Hereditary red blood cell disorders in middle eastern patients. Mayo Clinic Proc 76, 2001, 285-93.
[40]. Bolton-Maggs PH. The diagnosis and management of hereditary spherocytosis. Baillieres Best Pract Res Clin Haematol 13, 2000, 327-42.
[41]. Marchetti M, Quaglini S, Barosi G. Prophylactic splenectomy and cholecystectomy in mild hereditary spherocytosis: analyzing the decision in different clinical scenarios. J Intern Med 244, 1998, 217-26.
[42]. Bader-Meunier B, Gauthier F, Archambaud F, Cynober T, Mielot F, Dommergues JP, et al. Long-term evaluation of the beneficial effect of subtotal splenectomy for management of hereditary spherocytosis. Blood 97, 2001, 399-403.
[43]. Tchernia G, Bader-Meunier B, Berterottiere P, Eber S, Dommergues JP, Gauthier F. Effectiveness of partial splenectomy in hereditary spherocytosis. Curr Opin Hematol 4, 1997, 136-41.
[44]. De Buys Roessingh AS, de Lagausie P, Rohrlich P, Berrebi D, Aigrain Y. Follow-up of partial splenectomy in children with hereditary spherocytosis. J Pediatr Surg 37, 2002, 1459-63.
[45]. Graham HN. Green tea consumption, composition, and polyphenol chemistry.Preventive Medicine. 21, 1992, 334-50.
[46]. Vinson JA, Dabbagh YA. Effect of green and black tea supplementation on lipids,lipid oxidation and fibrinogen in the hamster: mechanisms for the epidemiological benefits of tea drinking. FEBS Letters. 433(1-2), 1998, 44-6.
[47]. Tijburg LB, Wiseman SA, Meijer GW, Weststrate JA. Effects of green tea, black tea and dietary lipophilic antioxidants on LDL oxidizability and atherosclerosis in hypercholesterolaemic rabbits. Atherosclerosis. 135(1), 1997, 37-47. *Nutrition Noteworthy*, 2(1,7), 1999.
[48]. Pearson DA, Frankel EN, Aeshbach R, German JB. Inhibition of endothelial cell mediated oxidation of low-density lipoproteins by green tea antioxidants. FASEB Journal. 10, 1996, A476
[49]. Zhang A, Zhu QY, Luk YS, Ho KY, Fung KP, Chen ZY. Inhibitory effects of jasmine green tea epicatechin isomers on free radical-induced lysis of red blood cells. Life Sciences. 61(4), 1997, 383-94.
[50]. Constable A, Varga N, Richoz J, Stadler RH. Antimutagenicity and catechin content of soluble instant teas. Mutagenesis. 11(2), 1996, 189-94.
[51]. Anderson JW, Diwadkar VA, Bridges SR. Selective effects of different antioxidants on oxidation of lipoproteins from rats. Proceedings of the Society for Experimental Biology and Medicine. 218(4), 1998, 376-81.
[52]. Yamanaka N, Oda O, Nagao S. Green tea catechins such as (-)-epicatechin and (-)-epigallocatechin accelerate copper-induced low-density lipoprotein oxidation in propagation phase. FEBS Letters. 401(2-3), 1997, 230-4.
[53]. Yokozawa T, Dong E. Influence of green tea and its three major components upon low-density lipoprotein oxidation. Experimental and Toxicologic Pathology. 49(5), 1997, 329-35.

[54]. Princen HM, van Duyvenvoorde W, Buytenhek R, Blonk C, Tijburg LB, Langius JA, Meinders AE, Pijl H. No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers.Arteriosclerosis, Thrombosis, and Vascular Biology. 18(5), 1998, 833-41.

[55]. Van het Hof KH, de Boer HS, Wiseman SA, Lien N, Westrate JA, Tijburg LB. Consumption of green or black tea does not increase resistance of low-density lipoprotein to oxidation in humans. American Journal of Clinical Nutrition. 66(5), 1997, 1125-32.

[56]. Imai K, Nakachi K. Cross-sectional study of effects of drinking green tea on cardiovascular and liver disease. BMJ. 310, 1995, 693-6.

[57]. *Dr. Murtadha Al-Shareifi e-Library* Lippincott's Illustrated Reviews: Pharmacology

[58]. *Lalita Kunlawong, Natsuda Ponwiang, Ubonwan Jaihan, Somdet Srichairatanakool, Chairat Uthaipibull, and Voravuth Somsak* Anti-Hemolytic Effects of Green and Black Tea Aqueous Extracts on Plasmodium berghei-Infected Mice Advanced Natural Products 1 (1–6), 2014.

[59]. Chonthida Thephinlap, Kanjana Pangjit, Maitree Suttajit1 and Somdet Srichairatanakool., Journal of Medicinal Plants Research Anti-oxidant properties and anti-hemolytic activity of *Psidium guajava, Pandanous odorus and Rhinacanthus nasutus 3, 2002.*