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Aquatic Pathogens are Subjected to Biosynthesis, Purification and Antibacterial Activity

Ajay Sinha [1], D. Chaudhary[2] and A.V Mukherjee[3]
[1]Department of Mechanical Engineering, Karunya Institute of Technology and Sciences, Coimbatore, INDIA.
[2]Department of Mechanical Engineering, Karunya Institute of Technology and Sciences, Coimbatore, INDIA.
[3]Department of Mechanical Engineering, Karunya Institute of Technology and Sciences, Coimbatore, INDIA.

Corresponding Author: drchaudhary1974@gmail.com

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ABSTRACT

The size and shape of the nanoparticles can be controlled by varying the ratio of metal salts to apiin compound in the reaction medium. The resultant nanoparticles were characterized by UV-vis-NIR, transmission electron microscopy (TEM), FT-IR spectroscopy, X-ray diffraction (XRD) and thermogravimetric analysis (TGA). The interaction between nanoparticles with carbonyl group of apiin compound was confirmed by using FT-IR analysis. Can drinking several cups of green tea a day keep the doctor away? This certainly seems so, given the popularity of this practice in East Asian culture and the increased interest in green tea in the Western world. Several epidemiological studies have shown beneficial effects of green tea in cancer, cardiovascular, and neurological diseases. The health benefits associated with green tea consumption have also been corroborated in animal studies of cancer chemoprevention, hypercholesterolemia, artherosclerosis, Parkinson's disease, Alzheimer's disease, and other aging- relateddisorders. Tea catechins extracted from green tea and tea extract have potential as defense against aging and neurodegenerative diseases caused by age-associated functional deterioration and neurodegeneration from an increase in free radical generation and oxidative stress such as cancer, Parkinson's disease, Alzheimer's disease, stroke, cardiovascular disease, and diabetes.

Keywords- Green tea, Cancer, Neuroprotection, Cardiovascular disease, Prevention, Epigallocatechin gallate, EGCG.

I. INTRODUCTION

Nanoparticles are usually referred to as particles with a maximum size of 100 nm. Nanoparticles exhibit completely new or improved properties compared to larger particles of the bulk material and these novel properties are derived due to the variation in specific characteristics such as size, distribution and morphology of the particles. Nanoparticles present a higher surface area-to volume ratio with decrease in the size of the particles. Specific surface area is relevant for catalytic activity and other related properties such as antimicrobial activity of AgNPs1,2,3. As the specific surface area of nanoparticles is increased, their biological effectiveness can also increase on the account of a rise in surface energy. Nanoparticles have a wide range of applications, as in combating microbes 4, biolabelling 5, and in the treatment of cancer 6. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods 7. There have been recent reports on phytosynthesis of silver and gold nanoparticles by employing coriander leaves 8, sundried Cinnamomum camphora leaves 9, phyllanthin extract 10, and purified apiin compound extracted from henna leaves 11. t is large sporadic resinous tree attains the maximum height of about 20-30m with maximum width of 150-190 cm. Bark is thick, rough dark brown longitudinally furrowed, within the furrows gum is exuded. Simple leaves with ultimate reticulate venation. Inflorescence is Axillary/ terminal panicles 12. Flowers white. Fruits with a woody pericarp with wings 2-3 times as long as capsule 13. S.tumbuggaia is a tree taxa with economic and medicinal values. Stem is used in marine yards as a substitute for pitch. The tree trunk is in use as flag poles for temples. The heart wood is similar to sal but much smoother and better for carpentry. The plant extracts used to cure ear-aches, external stimulant. Leaf juice is used as ear drops for children. The bark having anti ulcer activity 14. The stem is a source of resin, which is use as incense. The Resin used to cure duodenal ulcers and Amoebic dysentery. It is also used in indigenous medicine as an external stimulant and a substitute for Abietis; Resina and Pix Burgundica of European pharmacopoeias. Although this plant is considered as undesirable plant, but to the best of our knowledge we are the first to report its use in synthesizing silver nanoparticles, which can provide a new platform to this plant making it a value added tree for nanotechnology based medicine in future.

The World Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs considered being less toxic and relatively free from side effects. In general, herbal medicines are complex mixtures of different compounds that often act synergistically to exert their full beneficial effect on diabetes mellitus and other disease. Green tea compounds may influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic B cells, modulation of glucose release from liver, activation of insulin receptors (enhancing insulin binding) and glucose uptake in the insulin-sensitive tissues, and modulation of hepatic glucose output.

II. MATERIALS AND METHODS

Materials

Silver Nitrate ($AgNO_3$) was obtained from Sigma Aldrich and Green tea was collected from the local market of our institute.

Plant extract

1.3 g of green tea bag was left for 20 min in boiling water to get extract. The extract was cooled at room temperature and then filtered by using Whattman filter paper No. 1 (Figure 2).

Silver nitrate solution

Silver nitrate aqueous solution of 1 mM was prepared by dissolving appropriate amount of silver nitrate salt in suitable amount of distilled water and stored in amber color bottle.

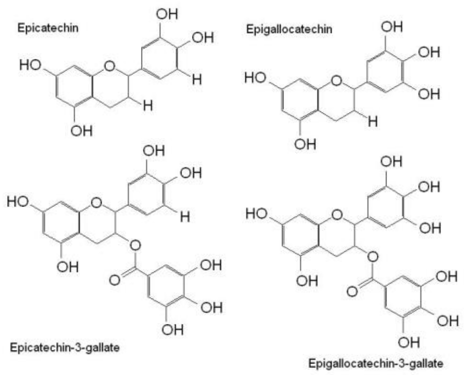

Figure 1: Epicatechin and tea flavins present in green tea

Figure 2: Green tea leaf

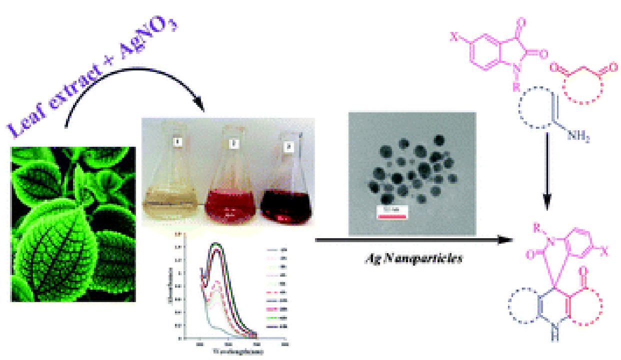

Figure 3: Synthesis of Ag-NP from reaction between $AgNO_3$ and green tea extract

Synthesis of silver nanoparticles

60 ml of green tea extract were taken with a pipette and diluted to 500 ml in a volumetric flask and hand-shaken to homogenize the dilution. The pH of the resulting solution was 6 and was make alkaline by adding a very small portion of potassium carbonate (K_2CO_3) until a pH=10 was reached. Then 20 ml of the $AgNO_3$ stock solution were added in a single shot (Figure 3).

Formation of silver nanoparticles

The color change was noted by visual observation in the Erlenmeyer flask which contains $AgNO_3$ solution with green tea extract.

UV-Visible spectroscopy

The reduction of silver ions in the colloidal solution was confirmed by UV-Visible spectroscopy. A small aliquot from Ag NPs was taken in a quartz cuvette and observed for wavelength scanning between 200 to 900 nm with distilled water as a reference. The UV-Vis absorption spectrum of the sample was performed in Perkin Elmer Spectrophotometer, at different time 5, 10, 15, 20, 25 min after addition of green tea extract on $AgNO_3$ solution.

Scanning electron microscopy

Surface morphology of silver nanoparticles was detected by scanning electron microscopy (SEM). The sample was prepared by centrifuging colloidal solution after 6 h of reaction at 14,000 rpm for 4 min. The pellet was redispersed in deionized water and again centrifuged. The process was repeated three times and finally washed with acetone. The purified silver nanoparticles were sonicated for 10 min for making the suspension. Thin films of the sample were prepared by dropping a very small amount of the sample on glass plates and then allowed to dry at room temperature.

The sample was kept under lamp until completely dry. The prepared sample was subjected to SEM analysis by using (SEM) with Zeiss 700 Scanning Electron Microscope (SEM).

Transmission electron microscopy

Shape and size distribution of the synthesized Ag nanoparticles were characterized by transmission electron microscopic (TEM) study. Few drops of Ag nanoparticle solution were dropped onto a TEM grid, and the residue was removed by a filter paper beneath the TEM grid. The prepared sample was subjected to TEM analysis was performed by using a JEOL JEM-2100F (JEOL, Tokyo, Japan) instrument operating at 120 kV, which has a diffraction pattern recorded at selected areas to determine the particle structure and size of silver nanoparticles at a 660 mm camera length.

FTIR spectroscopy analysis

For the Fourier transform infrared (FTIR) spectroscopy analysis, the residual solution was centrifuged at 10,000 rpm for 15 min. To remove any free biomass residue and the resulting suspension was redispersed in 1 mL sterile distilled water. There after the purified suspension was freeze dried to obtain dried powder. Dried Ag NPs were mixed with potassium bromide (KBr) and the spectra were recorded with a Perkin Elmer Spectrum (Perkin Elmer, Germany).

III. RESULTS AND DISCUSSION

The reduced silver ions were analysed by using the UV-VIS spectrophotometer. The absorption spectra of silver nanoparticles in the reaction mixture had an absorbance peak at 440 nm which coincided with the results obtained with mangrove plant, Rhizophora mucronata . A similar UV-Vis absorption spectra has been observed with silver nanoparticles synthesized from Acorous calamus rhizome extract and Terminalia chebula fruit extract. The obtained result was very close with silver nanoparticles synthesized by Cochlospermum religiosum extract and Pithophoraoe dogonia extract. A similar report was also reported in the literature by using plant extract with different concentration Musa balbisiana (banana), Azadirachta indica (neem) and Ocimum tenuiflorum (black tulsi) and Azadirachta indica.

The aqueous leaf extract and nanoparticle pellets were characterized through FTIR spectra analysis. Based on the absorbance bands different functional groups were identified. The FTIR spectrum of aqueous leaf extract of Mirabilis jalapa and green synthesized silver nanoparticles was shown in fig 2 and 3. The absorption spectra of extract indicated a peak position of 1053.13, 1465.90, 1483.26, 1516.05, 1639.49, 2358.94 and 3361.93 cm^{-1}. The colloidal solution of silver nano particles indicated a absorption peak at 1045.42, 1222.87, 1450.47, 1467.83, 1504.48, 1635.64 and 3358.07 cm^{-1}. The absorption peaks exhibited a measured shift in the position and their possible interaction indicated that some constituents contributed to the reduction of silver ions and stabilize the bio reduced silver nano particles.

The band at 1053.13 cm^{-1} and 1045.42 cm^{-1} denotes CO group which corresponds to the carboxylic acid. The band at 1465.90 cm^{-1}, 1483.26 cm^{-1}, 1516.05 cm^{-1} of extract and 1450.47 cm^{-1}, 1467.83 cm^{-1}, 1504.48 cm^{-1} of AgNPs corresponds to N-H bend. The band at 1639.49 cm^{-1} and 1635.64 cm^{-1} arises from C-O group of carbonyl. 2358.94 cm^{-1} band in extracts shows the presence of aldehydic C-H is stretching. The absorption band at 3361.93 cm^{-1} and 3358.07 cm^{-1} is a characteristic of alcoholic and phenolic group present in extract and AgNPs. Our report correlated with the previous reports on the presence of phenolic group, an aromatic compound in mirabilis aqueous extract was identified as possible bioactive compound in capping and stabilizing the silver nanoparticle of Mirabilis jalapa. It is also evidenced from the FTIR spectra of three medicinal plants Musa balbisiana (banana), Azadirachta indica (neem) and Ocimum tenuiflorum (black tulsi) , Citrus limon (lemon) , Eucalyptus oleosa , Azadirachta indica , Eucalyptus hybrida .

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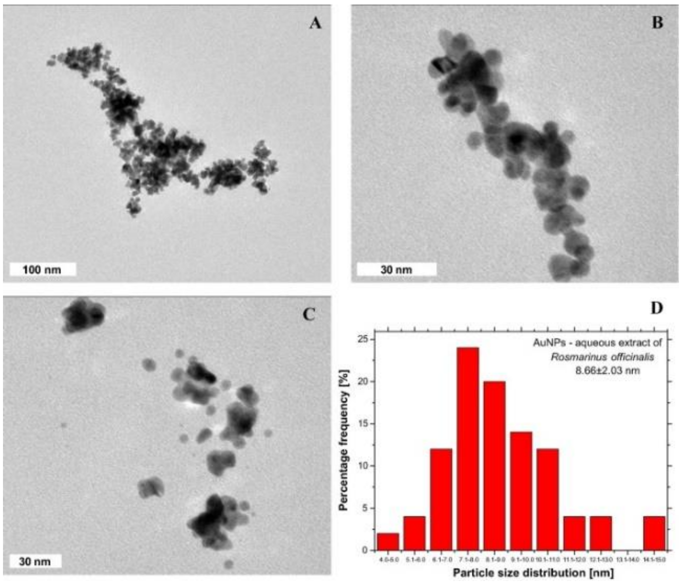

Figure 4: FTIR Spectrum of aqueous extract of Mirabilis jalapa

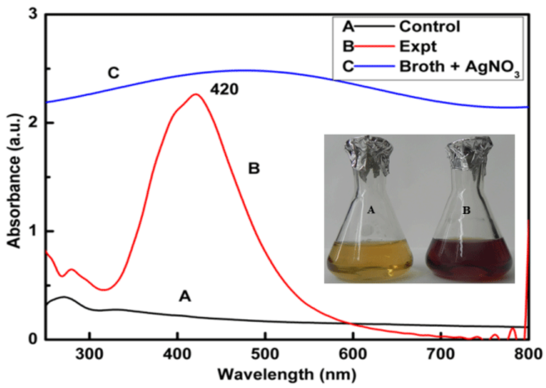

Figure 5: FTIR Spectrum of green synthesized silver nanoparticles

Phytochemical screening

The phytochemical analysis of aqueous leaf extract of Mirabilis jalapa was analysed for components such as alkaloid, Flavonoid, Tannins, phenol, Steroid, Saponins, and Glycosides. The aqueous leaf extracts of Mirabilis jalapa demonstrated to have maximum secondary metabolites like tannins, flavonoids, alkaloids and phenols. Steroid, saponins and glycosides are absent in aqueous extract of Mirabilis Jalapa. Saponins and alkaloids show its presence in silver nanoparticle synthesized leaf extract. Flavonoid, tannins, phenol, glycosides and steroids were not found in the silver nanoparticle synthesized leaf extract. The phytochemical results for the extract and silver nanoparticle studied are shown in Table 1.

Thus, the metabolites present in the leaf extract acted as electron donor and reduces silver ions into silver, formed by splitting of silver nitrate into silver and nitrate. This reduction leads to change in color of the reaction mixture. Consequently, the formation of nanoparticle was indicated by the brownish color of the aqueous solution following the excitation of surface plasmon vibrations.

Table 1: Phytochemical analysis from leaf extract of Mirabilis jalapa and its silver nano particles

S.NO	Phytochemical Test	Mirabilis jalapa extract	AGNP'S
1	Test for Tannins (Lead acetate)	+	-
2	Test for Saponin's (Foam froth)	-	+
3	Test for Flavonoids (Sodium hydroxide)	+	-
4	Test for Steriods (Salkowski)	-	-
5	Test for Alkaloids (Wagner and Hager)	+	+

6	Test for Phenol (Lead acteate)	+	-
7	Test for Glycoside (Bontrager)	-	-

(+) – indicates Presence (-) – indicates Absence.

Table 2: Inhibition zone of silver nanoparticles synthesized from the leaves of Mirabilis jalapa against bacterial species

S. NO	AGENT	CONC µl	ZONE OF INHIBITION (mm)				
			Salmonella	Bacillus	E.coli	Klebsiella	Proteus
1	ANP'S	10 20	11 13	11 13	-	13 15	13 14
2	ANTIBIOTIC	10	35	-	-	22	18
3	PLANT EXTRACT	10	-	11	-	10	10

Note: (-) indicates no effect

IV. ANTIBACTERIAL ACTIVITY

In the present investigation green synthesized silver nanoparticle, antibiotic and aqueous plant extracts were evaluated for antibacterial activity. The nanoparticle synthesis by using Mirabilis jalapa extract was found highly active against tested bacterial species at a concentration of 20 µl than 10 µl concentration. The results showed higher antibacterial activity against Klebsiella pneumonia.

In this study, the antimicrobial property of AgNPs was investigated by growing colonies on nutrient agar plates. Results obtained are shown in Table 2 and fig. 4. The inhibition zones obtained indicates maximum antibacterial activity of the prepared test sample. The zone of bacterial inhibition by AgNPs (20 µl and 10 µl) prepared from mirabilis leaf extract show maximum inhibition for Klebsiella pneumonia followed by Proteus sp., Salmonella sp., and Bacillus sp which may be concluded from the fact these particles had the smallest diameter to cross the cell membrane. 20 µl of silver nanoparticle exhibited a maximum zone of inhibition towards Klebsiella pneumonia (15mm). A moderate activity towards Proteus sp with a zone of inhibition of 14 mm. Salmonella and Bacillus showed a lower resistance towards AgNps with a zone of inhibition of 13mm. 10 µl concentration of AgNPs in turn exhibited equal antibacterial property as 20 µl. A similar report was produced by control (10 µl) against selected bacterias. Also, in comparison, plant extract (10 µl) showed prominent antibacterial activity towards Bacillus sp., (11mm), Klebsiella sp. (10mm) and Proteus sp. (10mm) only, while there was no such activity against Salmonella and E.coli. No signs of inhibition was obtained in case of E.coli by AgNps / control. . In a previous study, similar antibacterial activity was exhibited by silver nanoparticles synthesized from Acorus calamus against B.subtilis, B.cereus, and S.aureus. In another report, Cochlospermum religiosum leaf derived silver nanoparticle have been shown to display potent antibacterial activity against the selected pathogens as similar as our reports. Similarly results were obtained in the case of silver nanoparticles synthesized using Rhiophora apiculate and Eucalyptus camaldulensis.

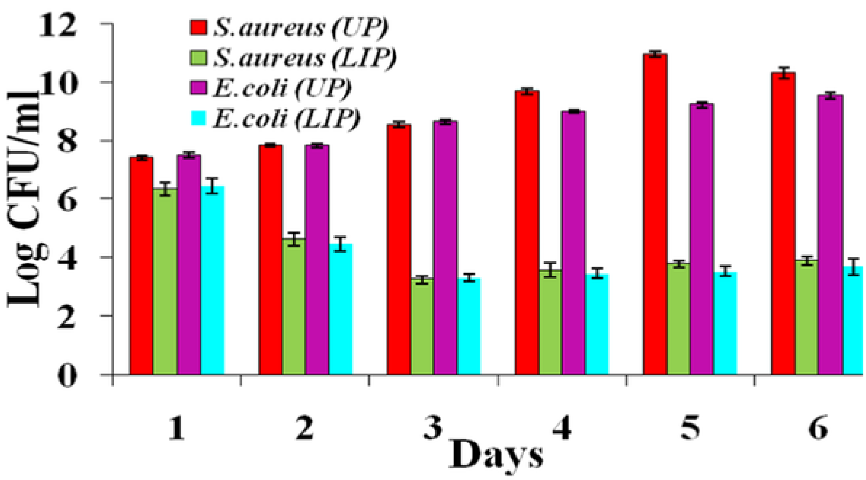

Figure 6: Antibacterial activity of Mirabilis jalapa plant extract (10µl), antibiotic (10µl) and silver nano particles (10µl and 20µl).

According to some workers, the basic interaction mechanism behind the inhibitory action of silver nanoparticles towards microorganisms may be due to the presence of positive charge nanoparticles and the negatively charged cell membranes of microorganisms [24,25,26]. At the initial stage of the interaction process, silver nanoparticles gets adhered on the wall of bacteria and this leads to accumulation of Ag nanoparticles in the bacterial membrane, causing alteration in permeability and resulting in cell death [27,28,29]. Consequently, membrane permeability gets distorted, due to progressive release of lipopolysaccharide molecules and membrane proteins. Danilczuk et al., 2006 reported that Ag nanoparticles are associated with the liberation of free radicals, which in turn damages the cell membrane. Similarly, Lok et al., 2006 reported that free silver ions cause interruption in energy production, in the form of ATP and DNA replication process. This result also coincides with [32, 33, 34].

Further, the present study proved that the biogenic nanoparticles synthesized using aqueous plant extract appears to be promising and as an effective antibacterial agent compared to plant extract against the bacterial strains selected for the present study.

V. HEALTH BENEFITS OF GREEN TEA

Anticancer Activity

Green tea polyphenols have been shown to have anti-mutagenic and anti-carcenogenic properties in vitro and in vivo. Green tea was found to activate intracellular antioxidants, inhibit precarcinogen formation and suppress angiogenesis and cancer cell proliferation in different tissues. A study conducted in Japan indicated that EGCG was able to suppress cell adhesion, an important step in the development of disease in multi-cellular organisms, through the inhibition of focal adhesion kinase (FAK) and insulin like growth factor I receptor (IGF-IR). The inhibition of FAK and IGF-IR was able to inhibit proliferation of pancreatic carcinoma cells. Another study by Mantena, et al, also demonstrated that orally administered green tea polyphenols were able to prevent ultraviolet radiation induced skin cancer. According to this study the mechanisms involved were activation and recruitment of cytotoxic T-lymphocytes to the cancer microenvironment and inhibition of angiogenic factors like matrix metalloproteinase (MMP)-2 and MMP-9 which was also showed by another mouse model study conducted by Vayalil and collogues. Other mechanism of green tea in cancer prevention includes activation of mitogen activated protein kinase (MAPK) path way by inhibition of enzymes such as the cytocromes P450 which are involved in the bio-activation of carcinogens, it involve in phase II detoxification, inhibition of urokinase activity, induction of apoptosis in already induced carcinomas, inhibition of cellular proliferartion and tumor progression. Through similar mechanisms green tea prevent many types of cancer associated with lung, colon, oseophagus, mouth, stomach, small intestine, kidney and mammary glands.

The Impact of Green Tea on Diabetes Mellitus

Diabetes mellitus is now considered to be a worldwide epidemic and without primary prevention, the epidemic will continue increasing. The pathology of diabetes mellitus is caused by reactive oxygen species that activate the non-enzymatic glycation of proteins (leads to structural and functional changes), the aldose reductase pathway (causes sorbitol accumulation) and oxidative stress (results in protein, DNA and lipid damage). The complications of diabetes mellitus, like retinopathy, nephropathy and neuropathy, are results of such pathologic mechanisms. But these complications can significantly be prevented or their occurrence can be delayed by strict control blood glucose level. The World Health Organization Expert Committee on diabetes recommended that traditional medicinal herbs considered being less toxic and relatively free from side effects. In general, herbal medicines are complex mixtures of different compounds that often act synergistically to exert their full beneficial effect on diabetes mellitus and other disease. Green tea compounds may influence glucose metabolism by several mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic B cells, modulation of glucose release from liver, activation of insulin receptors (enhancing insulin binding) and glucose uptake in the insulin-sensitive tissues, and modulation of hepatic glucose output.

Cardiovascular Diseases

As reviewed by Wolfram, several intervention studies have demonstrated that green tea catechins containing 200–300 mg EGCG will exert beneficial effects on cardiovascular and metabolic health by decreasing oxidative stress which participates in the pathogenesis of cardiovascular disease. A cohort study done by Kuriyama, et al, in Japan demonstrated that consumption of green tea was associated with reduced mortality due to cardiovascular risks. Another study from the same country has also showed a reduced incidence of stroke among individuals who consumed green tea. Mechanisms involved may include inhibition of angiotensin-converting enzyme activity by green tea polyphenols (when the enzyme is inhibited angiotensin, I will not be converted to angitoensin II and this in turn lowers the blood pressure of individuals), increased nitric oxide production by endothelial cells, and improved endothelium-derived nitric oxide bioactivity and suppress blood pressure.

Effect of Green Tea on Enhancing Insulin Activity

Elevated glucose concentration in blood promotes secretion of insulin from the β-cells of the islets of Langerhans in the pancreas, and insulin mediates the uptake of glucose in peripheral tissues including muscle, adipose tissue and kidney, promotes storage of glucose in liver as glycogen, and inhibits lipolysis in adipose tissue. A study conducted by Wu and colleuges in Taiwan, to assess the impact of green tea supplementation on insulin sensitivity showed the beneficial effect of green tea in diabetes mellitus. According to this study insulin binding capacity among rats that took green tea supplementation was significantly higher than the control groups. The study has also confirmed that the increase in insulin induced glucose uptake among those rats with green tea supplementation by in vitro analysis of adipocytes tissue. Another study by Yan, et al, also indicated that green tea catechins are able to ameliorate(improve) insulin resistance in adipose tissues by improving oxidative stress and this was demonstrated in their study by decreased serum reactive oxygen species and recovery of impaired insulin stimulated glucose up take in mice that took EGCG. However, a randomized controlled human trial indicated the absence of any significant difference in the insulin sensitivity, insulin secretion and glucose tolerance between those who took EGCG and the placebo group. Insulin enhancing property of green tea was also determined using rat epididymal adipocytes. In this study both insulin dependent break down of glucose to carbon dioxide and incorporation of glucose in to lipids were enhance by green tea, indicating it ability to potentiate insulin activity.

VI. CONCLUSION

Different studies reviewed in this manuscript showed that green tea has effects in carbohydrate metabolism so thatit can be a good candidate in the alleviation of diabetes mellitus. Inhibition of carbohydrate digestion and glucose absorption, stimulation of pancreatic cells to produce insulin, increasing insulin activity and actions on the liver to maintain glucose homeostasis are the mechanisms of green tea polyphenols to prevent diabetes mellitus. Both human and animal model studies also showed that green tea has different health benefits such as anti-cancer activity, prevention of neurodegenerative diseases, prevention of cardiovascular disorders, amelioration of lung injury, antimicrobial and antiviral activity and prevention of NAFLD. Some of the mechanisms involved in the prevention of these diseases include antioxidant activity of polyphenols, their involvement in different signal transduction and their ability to inhibit cancer proliferation and metastasis. Together with its health benefits dose dependant adverse effects of green tea were observed in the gastrointestinal tract, liver and nervous system. But these side effects are absent or minimum if present. Such advantage of green tea is increasing interests to use it as an alternative medicine for different types of chronic diseases. Hence, further researches on determination of effective therapeutics dose, mechanism of action and any side effects must be conducted.

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