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Research Paper

Chemopreventive, Neuroprotective And Cardioprotective Activities Of Lycopene And Its Formulations

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ABSTRACT
Lycopene belongs to a class of compounds called Carotenoids or tetraterpenoids. Various studies have been performed to unearth the potential benefits of Lycopene based on its Antioxidant and Anticancer Activities. Recent studies have also attributed to more promising activities of Lycopene such as Anti Inflammatory, Anti Diabetic, and Antiepileptic Properties. Given the widespread use of tomatoes in our diet, we attempt to emphasize the potential benefits of Lycopene based on its Anticancer, Antioxidant, neuroprotective and cardioprotective Activity with an overview regarding its formulation. Various databases like PubMed, ScienceDirect and Google Scholar were screened for relevant studies on the anticancer and cardioprotective implications of Lycopene including its formulations. It was found that Lycopene directed various molecular mechanisms against cancer and cardiac complications. The antioxidant activity of Lycopene was found to contribute largely to the potential benefits of the constituent. Various studies were also indicative of the emerging trend on novel formulations of lycopene. These findings can be used to improve the potential scope of lycopene as a compound of nutraceutical and pharmaceutical significance, in the management of cancer and other cardiac ailments.

Keywords *Lycopene Anti-cancer Cardioprotective Formulation*

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I. INTRODUCTION

Lycopene belongs to a class of Polyphenolic compounds called Carotenoids or Tetraterpenoids. These compounds attribute to the yellow, orange, or Red-colored pigmentation in various fruits [1]. They are formed from eight Isoprene units, made of forty carbon atoms. There are over 1100 known carotenoids, of which Lycopene is the most prominent compound found majorly in Tomatoes (*Lycopersicon esculentus*) belonging to the Solanaceae family. Two compounds, Isopentenyl diphosphate, and Dimethylallyl diphosphate form the building blocks of Carotenoids[2].

Lycopene has been widely studied for its Anticancer and Antioxidant Activities and its various mechanisms have been elucidated. Lycopene has been found to Inhibit Cancer Progression and play a protective role in various cancers by regulation of several key biomolecules and Cell Cycle modifications [3]. For example, high dietary intake and circulating levels of Lycopene have protective effects against prostate cancer, in a dose-dependent way. Lycopene has also been extensively studied for its Inhibitory effect on the proliferation and progression of colorectal cancer cells [4].

The Antioxidant activity of lycopene has also been enormously studied. It is the second most potent antioxidant after astaxanthin, among the family of Carotenoids. It is a major deactivator of Reactive oxygen species (ROS) [5]. Lycopene exhibits a high quenching rate of singlet oxygen, which directly influences its Antioxidant activity. The Quenching effect of Lycopene is related in part to the opening of the beta-ionone ring to an open-chain form. This activity of Lycopene is directly linked to the preventive role of the compound in Atherosclerosis as oxidation stress can cause damage to the endothelial cells, an important etiology for the disease[6].

Studies have also outlined the preventive role played by Lycopene in Atherosclerosis. Lycopene plays a role in scavenging Hypochlorous Acid, a acid that contributes to the pathology of atherosclerosis, Inflammatory disease, Respiratory stress, acute vasculitis, and cancer[7]. Lycopene also prevents Cardiovascular

complications by modulating Low-Density Lipoprotein oxidation. Oxidized LDLs are highly atherogenic due to foam cell formation[8].

Given the widespread potential benefits of Lycopene on human health, studies have been performed extensively on its formulation into novel dosage forms to achieve targeted delivery and retain maximum bioavailability[9]. For instance, the development of Lycopene-rich microemulsions made way for water-based food systems such as beverage and bakery products. Lycopene can also be formulated by trapping in a protein (Lactolycopene) to increase its absorption and bioavailability in contrast to unprocessed tomatoes. Lycopene can also be formulated into nanodispersions and Solid Lipid nanoparticles. Lipid-based nanoformulation of Lycopene has been found to improve oral delivery[10].

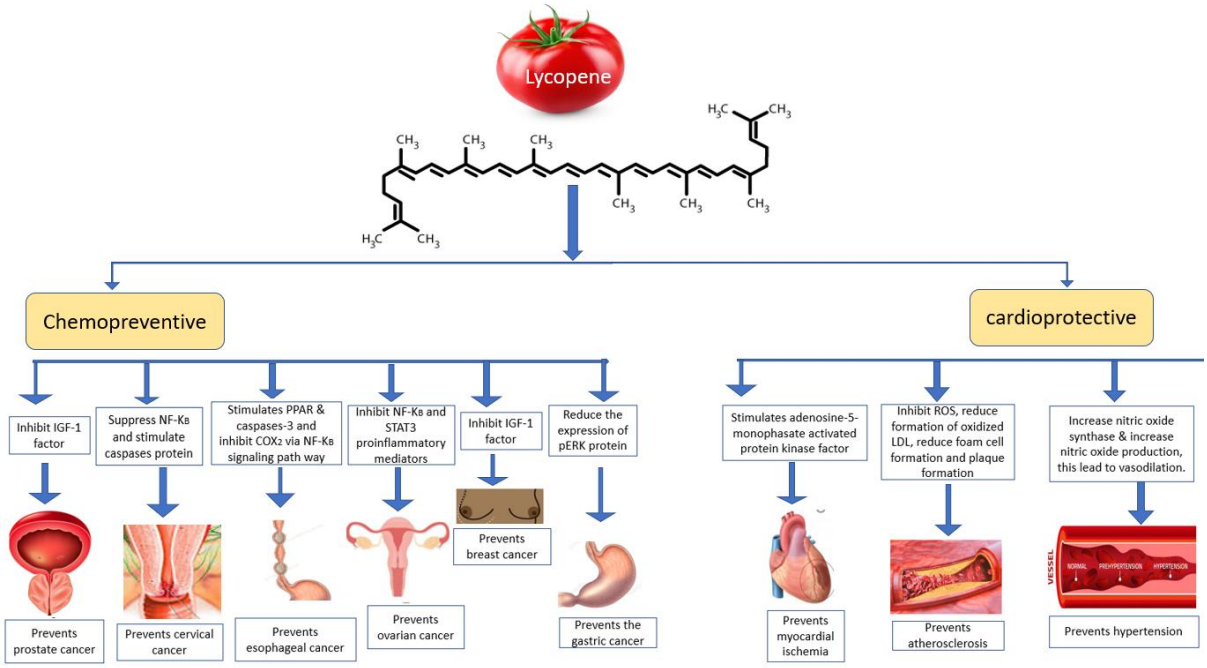

Fig:1. Structure of lycopene and its protective role in various organs

1.1 BIOCHEMISTRY OF LYCOPENE

Lycopene belongs to a large family of compounds called Carotenoids. Carotenoids are formed by the linkage of two C20 geranyl-geranyl diphosphate molecules. It consists of a polyisoprene structure, made of a long conjugated chain of double bond and a near bilateral symmetry around the central double bond[11]. Carotenoids are divided into two types, a) Provitamin A and b) Non-provitamin A. Provitamin A includes compounds like beta-carotene, alpha-carotene and beta-cryptoxanthin. Carotenoids are further classified on the basis of Functional groups as a) Xanthophylls (contain oxygen as functional group) and b)carotenes (contains only a parent hydrocarbon chain without any functional group)[12]. Xanthophylls include compounds like Lutein, and Zeaxanthin, whereas Carotenes include compounds like alpha-carotene, beta-carotene and Lycopene[13]. Apart from these classes, there are another class of compounds called Apocarotenoids that are derived from Carotenoids by oxidative cleavage using carotenoid cleavage deoxygenases. Carotenoids are hydrophobic molecules exhibiting minimal solubility in water, they act in hydrophobic areas of the cell. Lycopene, a provitamin A carotenoid, is a phytochemical found primarily in tomatoes and tomato-based foods[14]. It's a tetraterpene made up of eight isoprene units and eleven double linear linkages. Lycopene attributes to the red colouration of certain fruits and vegetables, in some plants. It is also an intermediate of Carotenoid biosynthesis. It should be noted that the human body cannot synthesise lycopene. As a result, it must be included in one's daily diet[15]. Lycopene is predominantly stored in the liver, adrenals, and prostate. Furthermore, it can be found in lower concentrations in other body parts (for example, the brain and skin). Ageing can reduce lycopene bioavailability, and some pathological conditions can reduce lycopene bioavailability such as cardiovascular diseases. As a result, its supplementation—via various means, such as pasteurised watermelon juice—has been proposed to increase its circulating serum level in needy populations[16].

Lycopene (C40H56), a hydrocarbon carotenoid, has an acyclic open chain structure with 13 double bonds that undergo isomerization and different cis isomers, such as 5, 9, 13, and 15, that have been observed in plants and blood plasma[17]. Lycopene is naturally found in all trans isomers, but the cis isomers are the most common type in tissue and plasma. This occurs during food preparation, processing, storage, and transportation,

as well as during metabolism in the body[18]. The conversion of cis to trans isomerization occurs in enterocytes, the liver, and the stomach. Scavenger receptors CD36 and B1 aid in lycopene absorption in the intestine. In the enterocyte, partial metabolism can be seen, which is aided by two enzymes: 150 -oxygenase-1, -carotene-15, which is linked to blood lycopene levels, and 100 oxygenase-2, -carotene-9. Furthermore, research on labelled lycopene molecules is limited[19]. For example, in a study on 14C-labeled lycopene (92 percent trans-lycopene), Ross et al. discovered that the trans-lycopene was completely isomerized (5-cis, 9-cis, 13-cis, and 15-cis lycopene isomers) after dosing and quickly converted into polar metabolites excreted in the urine[20]. The rapid exclusion of 14CO2 indicated that the ingested lycopene had been sufficiently oxidised. 13C-labeled lycopene was used in the compartmental model study. There were no significant differences in the bioavailability of cis and trans-lycopene (24.5 vs. 23.2%, respectively)[21]. Furthermore, it was established that post-absorptive trans-to-cis isomerization alters the isomeric profiles of tissues and plasma. The all-trans-isomer is the most thermodynamically stable lycopene configuration. Isomerization from the trans-isomer to several mono- or poly-cis types can be caused by light, heat, or a variety of chemical reactions[22].

II. ANTICANCER ACTIVITY

The anticancer activity of Lycopene has been broadly studied and its preventive, as well as protective role against many cancers, has been well established. Lycopene has been found to be effective against Prostate Cancer, Ovarian Cancer, Breast Cancer, Endometrial Cancer, Cervical Cancer, etc[23]. In this paper, we attempt to emphasize and provide a summary of the various molecular targets directed by Lycopene against various forms of cancer.

2.1 Oesophageal Cancer

Lycopene was found to inhibit the incidence of Oesophageal Cancer in F344 rats by Promoting Apoptosis based on PPAR, COX-2, and Caspase-3. PPAR is a protein that forms a central part in various signal transduction pathways, regulating various physiological and pathological activities[24]. PPAR promotes tumor cell apoptosis, induces cell differentiation, inhibits angiogenesis, and blocks the cell cycle. Lycopene was hypothesized to act as an agonist of PPAR to inhibit inflammation and induce apoptosis. COX-2 is highly expressed in malignant cells and is believed to participate in the development of tumors, by promoting cell differentiation and inhibiting apoptosis. The anti-inflammatory activity of Lycopene down-regulates the expression of COX 2 protein via suppressing the NFkB signalling pathway. PPAR also induces apoptosis by inhibiting COX 2 [25]. Caspase 3 is the most crucial protein in the apoptosis pathway. It is an effector caspase present in the nucleus and cytosol that promotes apoptosis. Lycopene also inhibits inflammation of Oesophageal cancer based on PPAR and NF-kB Signalling [26]. The NFkB is a nuclear transcription factor that binds to the enhancer element of the immunoglobulin kappa light chain of activated B cells. The activation of the NFkB signaling pathway can induce the expression of inflammatory cytokines, chemokines, and their receptors. The accumulation of Proinflammatory cytokines adds up to the pro-tumorigenic microenvironment, which is critical for tumor initiation and progression[27].

Another study also demonstrated the suppressive activity of lycopene in human oesophageal squamous carcinoma cell line EC109 based on PPAR signalling pathway and modulating the expression of p21, cyclin D1 and COX-2 expression[28]. Crocetin, a carotenoid which is the principal active compound in Saffron has also been reported to Exhibit anti-cancer effects in human oesophageal squamous cell carcinoma KYSE-150 cells. Further, a meta-analysis of carotenoid intake and Oesophageal cancer suggested that a higher intake of beta-carotene, alpha-carotene, Lycopene, and beta-cryptoxanthin is associated with a lower incidence of Oesophageal Cancer[29].

2.2 Cervical Cancer

Lycopene sensitizes human Cervical Cancer cells (HeLa) to Cisplatin via Inhibition of Cell Viability, Up-regulation of Bax Expression, Downregulation of Bcl-2 Expression, Suppression of Nf-kB mediated Inflammatory responses, and modulation of Nrf2 mediated Oxidative stress [30]. Bcl-2 and Bax are two proteins that play a key role in apoptosis. Apoptosis, a clearance mechanism, can be defined as a "biochemical and morphological cellular change that occurs via caspase-mediated pathways in response to fatal stimuli". Apoptosis is mediated through two main pathways, the intrinsic and the extrinsic pathways which play a role in carcinogenesis regulation. The intrinsic pathway is mediated by the activation of Caspase 9 which occurs through the disruption of mitochondrial membrane and the sequential release of cytochrome C [31]. Mitochondrial membrane stabilization is warranted through proapoptotic (Bcl2/ Bcl XL) and antiapoptotic Bax Proteins. The viability of cells were determined by MTS assay and the expression of relevant proteins was determined by western blotting. It was found theta lycopene significantly increased expression of Bax protein and downregulated the expression of Bcl-2 protein in HeLa cells ($P<0.0001$ and $P<0.0001$ respectively) [32].

2.3 Gastric Cancer

ROS have been implicated in the progression of several diseases including cancer. ROS causes severe cellular injury and promotes tumor metastasis, angiogenesis, and invasion. Antioxidants can prevent such complications by effectively scavenging the ROS and nullifying their effects of causing carcinogenesis [33]. Our body boasts of antioxidant enzymes like glutathione (GSH), Glutathione Peroxidase (GPx), Glutathione-S-Transferase (GST), that help maintain the homeostasis surrounding the concentration of ROS in the body. Known for its excellent Oxygen quenching capacity among the family of Carotenoids, Lycopene is effective in decreasing Oxidative damage by activating and stimulating antioxidant enzymes such as GSH, GPx, and GST[34].

ROS can also cause serious manifestations that result in the progression of Carcinogenesis like inhibition of apoptosis of cancer cells and inducing Proliferation of malignant cells. Detailed mechanisms relating to such pathways and the preventive role of Lycopene in them have been well studied[35].

The Extracellular signal-regulated Kinase (ERK) signalling pathway is considered to be a key player in cell proliferation, differentiation, cell survival, and motility. It is one of the most important signalling pathways that connect the membrane receptors to the nucleus[35]. The pathway also involves cell cycle checkpoints and mitosis. Any aberrant changes or activation of the pathway manifests tumor development and progression[36]. Therefore, this pathway has been actively implored upon for molecular targets in cancer treatment. The prominent reaction of the pathway involves phosphorylation of various downstream components resulting in its various cellular functions. Lycopene was found to intervene and act at various stages in the pathway primarily attributing to its chemopreventive role in Gastric cancer[37]. Lycopene inhibits the phosphorylation of ERK proteins, resulting in reduced expression of pERK proteins in HGC-27 cells. Lycopene was also found to increase the concentration of HGC-27 cells in the G0-G1 phase and decrease the concentration in the S phase which is indicative that Lycopene inhibits cell proliferation by preventing DNA replication and protein synthesis[38].

2.4 Ovarian Cancer

An imbalance between the production and elimination of ROS causes oxidative stress, leading to Chronic Inflammation, resulting in the transformation of a Normal Cell to a Tumor Cell. Lycopene significantly reduced the expression of NF-kB attributing to its Chemopreventive role in Ovarian Cancer[39]. NF-kB is a key Pro-Inflammatory transcription factor. The expression of STAT3, another key Pro-Inflammatory mediator was found to be downregulated in Ovarian Cells on the administration of lycopene[40]. The study was performed on laying hen (*Gallus domesticus*) animal model since it has the optimal capacity to provide the most relevant pre-clinical model. It is also the only non-human animal that develops ovarian cancer spontaneously at a higher incidence[41]. Animals were divided into three groups, 50 hens were randomly assigned to each group. The control group was administered with 0mg of lycopene per kg of diet, the second and third groups were administered with 200 or 400mg of lycopene per kg of diet. Lycopene administration was performed for 12 months after which various tests were performed including necropsy, and histopathological evaluation. Western blot was performed on protein lysates obtained from ovarian tissue. The results showed that lycopene supplementation significantly reduced ovarian tumor incidence ($P< 0.01$). There was also a marked reduction in the size ($p< 0.004$) and number ($p< 0.005$) of tumors. Detailed molecular analysis revealed a reduction in the levels of NF-kB and an increase in the expression of nuclear erythroid factor 2 and its target protein heme oxygenase 1. Lycopene supplementation was also found to decrease the expression of STAT 3[41].

Another study showed that Lycopene was able to reduce ovarian tumor growth and intraperitoneal metastatic load. The study was performed in a bioengineered 3D cancer model, based on Hydrogels as human ovarian cancer cell (OV-MZ-6) delivery vehicle. The OV-MZ-6 cells were extracted from malignant ascites drained from a patient suffering from advanced cerous cystadeno-carcinoma[42]. The Animals used in the study were divided into two groups, one to establish the preventive aspects of Lycopene and the other to understand its treatment aspects. Animals in Preventive group, were administered with Lycopene dispersion and placebo, 2 weeks prior to implantation of Cell seeded hydrogels into animals. The regimen for animals in treatment group was started 4 weeks after surgery. The animals were administered with Lycopene, placebo, paclitaxel (taxol), lycopene+taxol, carboplatin (platin), lycopene+platin, taxol+platin, lycopene+taxol+platin. Lycopene and placebo were administered at a dose of 15mg/kg daily through oral gavage. Taxol (10mg/kg) and carboplatin (15mg/kg) were given intraperitoneally every two weeks in an alternating fashion[40]. Eight weeks post-surgery the animals were sacrificed, the tumor and metastatic tissues were removed, weighed and processed. The tissues were then subjected to qRT-PCR and Immunohistochemical analysis. The later was performed using a human specific antibody against nuclear mitotic apparatus protein 1 (NuMA). The neoplastic treatment and preventive regimen tested positive with the antibody. Lycopene prevention regimen was found to decrease intraperitoneal metasatic load, cancer related factors and CA-125 upon BLA analysis. The anti-tumorogenic effects were established by downregulation of ITGB-1, ITGA-5, MMP9, FAK, ILK, and EMT marker, all of which play an integral role in disease progression[40].

Meta analysis of the association between dietary lycopene intake and ovarian cancer in post-menopausal women resulted in an insignificant inverse relationship wherein a 3.7% reduction in the risk of ovarian cancer was observed compared to lycopene intake after adjusting to potentially confounding factors[43]. One more study showed that Lycopene reduced the proliferation of ovarian cancer cell line SKOC3 in-vitro. The study also indicated enhanced apoptosis of the cancer cells in response to lycopene. The results were confirmed by MTT assay to determine the proliferation of cultures treated with different concentrations of lycopene and Flow cytometry was performed to determine apoptosis. The study indicated that enhanced apoptosis was due to the ability of lycopene to increase the expression of pro-apoptotic factor Bax and reduced expression of anti-apoptotic factor Bcl-2[39].

2.5 Breast Cancer and Prostate Cancer

Lycopene is also believed to exhibit a chemopreventive role in breast cancer, by inducing cell cycle arrest and inhibiting IGF-1 induced cancer cell progression. IGF 1 is especially associated with an increased risk for Breast cancer in post-menopausal women[44]. However, despite its promising mechanisms against breast cancer, a study trying to correlate dietary and plasma Lycopene and other beta carotenoids against breast cancer failed to show any correlation[45].

High levels of Insulin-like Growth factor (IGF-1) is a potential risk factor to breast and prostate cancer. Lycopene interferes with cell cycle progression and IGF-1 signalling in Mammary (MCF-7), Lung (NCI-H226), and Prostate (PC-3) cancer cells. IGF-1 can induce the proliferation of many different cancer cell lines[46]. Lycopene was also found to inhibit the proliferation of MCF-7 Breast cancer cell line in a dose-dependent manner while inducing apoptosis through increased expression of p53 and Bax mRNAs in MCF-7 cells. The p53 is a tumor suppressor gene that regulates the balance between cell proliferation and apoptosis[47]. The level of p53 is overexpressed in various cancers. The effects of Lycopene were also studied on three different subtypes based on the expression of ER, PR, and HER 2. It was found that Lycopene possessed a chemo-preventive role in ER-negative, HER 2 positive, and triple-negative breast cancer. Lycopene was also found to arrest the G0-G1 cell cycle phase and reduce the S phase in Prostate cancer cell lines LNCAP and PC3[48].

III. ANTIOXIDANT ACTIVITY

ROS are compounds that contain oxygenated atoms with unpaired electrons (free radicals). These free radicals are highly reactive and actively participate in chemical reactions and are responsible for increased oxidative stress[49]. Oxidative stress is the major cause of the occurrence of several cancers. ROS are released as a result of physiological stress like exposure to ultraviolet radiation, ionization radiation, or high temperature, or due to metabolism of exogenous compounds like drugs, or they get released as a result of immune function[50]. However, about 90% of ROS are released into the cell through cellular respiration which takes place in the mitochondria of a cell[51].

The most commonly occurring ROS in a cell is the hydroxyl group (OH) and superoxide group(O2)[52]. Due to their short lifetime, the OH group causes immediate and extensive damage to the cells. It is important to note that ROS should be maintained in adequate amounts because they play a major role in regulating gene expression, cell signaling pathways, alongside activation of proteins and enzymes[53]. If the ROS is present in lesser amounts, the normal functioning of a cell gets interrupted. However, if their concentration increases it may lead to unfavorable consequences like damage to the DNA, chromatin, protein, lipid membrane, cytoskeleton, enzyme inactivation and may ultimately lead to apoptosis[54]. It, therefore, becomes crucial to maintain a balance between the production and elimination of ROS. Antioxidants can help prevent such complications arising due to Oxidative stress caused by an imbalance between the production and elimination of ROS[51].

Lycopene which is a phytochemical, non-pro-vitamin A (intermediate of carotenoid) is an excellent antioxidant[55]. It is a tetraterpene compound with eight isoprene units and eleven conjugated double bonds. This conjugated system shows an excellent capacity for quenching the ROS. The strength for antioxidant compounds to quench ROS are of the order,

lycopene > tocopherol > cryptoxanthin > zeaxanthin = β carotene[52].

Another mechanism for the anti-oxidant activity of lycopene is attributed to the activation of anti-oxidant enzymes such as GSH, GPx, and GST. Lycopene and other carotenoids were also found to induce the expression of Phase II enzymes contributing to its anti-oxidant activity[56]. The mechanism involves the activation of ARE transcription system. The coordinated action of Phase II enzymes like NAD(P)H:quinone oxidoreductase (NQO1) and γ-glutamylcysteine synthetase (GCS) is mediated through cis regulatory DNA sequences located in the promotor or enhancer regions known as Antioxidant Responsive Elements(ARE)[23]. The major ARE activating transcription factor Nrf2 plays a prominent role in the induction of anti-oxidant and detoxifying genes. Under Basal conditions the Nrf2 remains in the cytoplasm bound to its inhibitory protein keap1[52]. However, when acted upon by an inducer, it dissociates from the inhibitory protein and translocate to

the nucleus. The study demonstrated the antioxidant activity of lycopene in part to its role in activating the ARE transcription system, resulting an increase in the expression of phase II enzymes and the nuclear translocation of Nrf2 transcription factor[5].

Lycopene's antioxidant property is 10 times more than tocopherol (Vit E). Many studies have revealed that there is a inverse relationship between lycopene serum concentration and cancer[57]. As lycopene is not produced in the human body it should be taken through diet regularly, it gets absorbed through the stomach and stored in the liver, prostate glands, and adrenals[58]. Small amounts of lycopene are also found in the brain and skin. Generally, the concentration of serum lycopene decreases with age due to the presence of cardiovascular diseases. Lycopene also plays a key role in decreasing the platelet adhering proteins, resulting in reduced plug formation in the blood vessels[4]. The antioxidant property lycopene contributes extensively in reducing the incidence of skin, liver, prostate, ovary, breast, and colon cancers[59]. Lycopene also exhibits other key properties including antiepileptic, antidiabetic, anti-atherosclerotic activity. It also reduces the incidence of mouth ulcers and helps in maintaining dental hygiene[8].

TABLE -1: Anticancer activity of lycopene

Oesophageal Cancer	Promotes apoptosis based on PPAR, COX 2 And Caspase 3. Inhibits Inflammation based on PPAR and NFkB Signalling.
Cervical Cancer	Inhibition of Cell Viability, Upregulation of Bax expression, Downregulation of Bcl-2 expression, Suppression of NFkB mediated inflammatory responses Modulation of Nrf 2 mediated oxidative stress
Gastric Cancer	Stimulation of Anti-oxidant enzymes GSH, GST, GPx. Inhibition of ERK Signalling Pathway Cell Cycle Arrest in the G0-G1 phase of HGC-27 Cells.
Ovarian Cancer	Reduces the expression of Pro-Inflammatory transcription factors, NFkB and STAT 3. Downregulation of ITGA1, ITGB5, ILK, FAK, MMP9, EMT markers.
Breast and Prostate Cancer	Inhibits IGF-1 induced Cell cycle progression, Inhibits proliferation of MCF-7 Breast cancer cell line Induces Apoptosis through increased expression of p53 gene and Bax mRNAs in MCF-7 Cell line. Cell cycle arrest in the Prostate cancer cell lines LNCAP and PC3

IV. NEUROPROTECTIVE ROLE OF LYCOPENE

The major cause for neurological disorders are associated with accumulation of aluminum in the hippocampus region. This is the region where memory and cognitive functions are carried out. Aluminum (Al) is primarily present as insoluble mineral (i.e. bauxite)[60]. When this bauxite come in contact with acid rains aluminum get discharged as soluble mineral into the soil, from where it enters the plants. When human beings consume plant foods frequently, the aluminum present in that plants get accumulated in them[61]. This aluminum disturbs the balance between the ROS and antioxidant enzymes and also reduce the expression of

nuclear factor erythroid-2 related factor 2 (Nrf2) gene. This gene is involved in neuroprotective function against the increased ROS. When this gene is not expressed it contributes to neuron damage[62].

The neuroprotective function of lycopene was experimentally proven by cao 2019. In their study they took male wistar rats and divided the rats into 4 groups. Group -1 as control where only vehicle is administered. Group-2, aluminum is administered in the form of Alcl3. Group-3 was administered with Alcl3(150 mg/kg) and lycopene (4mg/kg)[63]. Group -4 with Alcl3(4 mg/kg) and lycopene (4mg/kg). From these groups 6 rats were selected randomly and morris water maze is conducted, the time spent by each rat in swimming was noted. The escape latency of group 1, 3 and 4 is more than group 2. The rats were then sacrificed by administering anesthesia (sodium pentobarbital) and brains were separated. This brains were divided into two halves[63]. One half of the Brain was fixed with 10% formalin and stained with hematoxylin and eosin (HE stain) for measuring Al content in hippocampus. Staining showed that there is clear regular arrangement of neurons in control and lycopene treated groups showed normal arrangement as that of control with few necrotic spaces. Whereas Alcl3 treated group showed irregular arrangement of neurons with pathological lesions. The second half is frozen rapidly in liquid nitrogen and stored at -80^{o}c to check Al content in hippocampus using graphite furnace atomic absorption spectrophotometry[63].

By using real time polymerase chain reaction (RT-PCR) the levels of interleukins -1β (IL-1), Tissue necrotic factor 2(TNF-α), superoxide dismutase (SOD-1), glutathione cysteine ligase catalytic subunit (GCLC) in hippocampus using trizol reagent were measured. The result shows, there was high levels of IL-1, TNF-α, SOD-1, GCLC levels in Alcl3 treated group than lycopene treated group. This shows that proinflammatory mediators play a major role apoptosis pathway[63]. Oxidative stress is measured by using commercial kits which measure the levels of malondialdehyde (MDA), glutathione (GSH) superoxide dismutase activity, 8-hydroxy-2-deoxy guanosine(8-OHdG) levels in hippocampus. Result revealed that there are high levels of MDA, 8-OHdG levels in Alcl3 treated group than lycopene treated group. The study suggests the role of Lycopene as a Neuroprotective[63].

V. PROTECTIVE ROLE OF LYCOPENE IN CARDIOVASCULAR DISEASES

Globally, cardiovascular diseases are the leading cause of morbidity and mortality. According to the WHO, an estimated 17.9 million people have died from CVDs contributing to 31% of global deaths in 2016[64]. The various factors that predispose to cardiovascular diseases include physical inactivity, unhealthy diet, obesity, and harmful use of alcohol. The physiological manifestations that contribute to cardiovascular complications include oxidative stress that results in atherosclerotic plaques, inflammatory mediators, reduced endothelial function, and atrial stiffness, all of which impair vascular health[65]. Given the widespread prevalence of CVDs, it is observed that more than half of such cases can be dealt with, by addressing behavioral risk factors that include diet and lifestyle modifications[66]. Lycopene which is well known for its excellent oxygen quenching capacity and its abundance in our diet can serve as an ideal molecule that could prevent cardiovascular complications[67].

5.1 Anti-Atherosclerotic Activity

Several studies have indicated the ability of lycopene to reduce oxidative stress, consequently inhibiting the concentration of pro-inflammatory mediators like cytokines, Interleukins, Tissue Necrotic Factors, and C- reactive proteins[7]. Lycopene also acts as a precursor for various oxidative cleaved products like APO lycopene (APO-8 & 12), which are involved in the overexpression of antioxidant and cytoprotective enzymes. It is also involved the regulation of PCSK9 which is known to combine with the LDL receptors and block them[68]. This will hinder the ingestion of LDL (low-density lipoproteins) from the extracellular fluid into the cells and increase the LDL concentration in blood plasma, which is a major predisposition, for the formation of atherosclerotic plaques[8].

Lycopene downregulates the expression of PCSK9 which contributes to decreased LDL concentrations in the systemic circulation. The anti-atherosclerotic activity of lycopene is due to inhibition of vascular smooth muscle cells (VSMC) proliferation and foam cell formation. It inhibits the VSMC from entering the S phase[69].

5.2 Pathophysiology of ROS Induced Atherosclerotic Plaques

The oxygen generated by vascular smooth muscle cells oxidizes the LDL, which accumulates in the atherosclerotic lesions. These oxidized LDLs subsequently produce more amount of ROS in the smooth muscle cells[70]. The Macrophages present in the muscular cells prompt to engulf the oxidized-LDL which results in the formation of foam cells in vascular intima. The foam cells further manifest as atherosclerotic plaques, resulting in impaired blood flow[71].

5.3 Anti-Hypertensive Activity

Oxidative stress can contribute to endothelial dysfunction by inhibiting the activity of Nitric Oxide Synthase[69]. Lycopene inhibits oxidative stress and increases the activity of NO synthase, which catalyzes the formation of nitric oxide. Nitric oxide in turn Improves endothelial-dependent vasodilation, contributing to reducing hypertension[72].

5.4 Anti-Ischemic Activity

Dysfunction of the endoplasmic reticulum can lead to "Endoplasmic reticulum stress", a serious manifestation that contributes to Ischemic/reperfusion injury. Lycopene protects cardiomyocytes by alleviating ERS via adenosine 5-monophosphate-activated protein kinase stimulation[73].

VI. LYCOPENE FORMULATIONS

Though lycopene has established potential health benefits such as chemopreventive and chemotherapeutic efficiency, it suffers from serious drawbacks like enhanced lipophilicity, poor solubility, and absorption that attributes to its decreased bioavailability[74]. Therefore, lycopene has been designed into different formulations to enhance delivery, improve absorption and bioavailability. With the potential benefits of Lycopene well studied, research has shifted to focus on novel formulations of the constituent that could contribute to enhancing its activity[75].

Lycopene has been formulated into smaller drug formulations such as nanodispersions and microemulsions that are traditionally known for targeted drug delivery. Lycopene has also been combined with proteins to enhance drug delivery that subsequently increases its bioavailability[76]. For Instance, a food-grade formulation, lactolycopene where lycopene was entrapped with whey proteins was found to enhance bioavailability. It was found that Lycopene from fresh, raw tomatoes and tomato juices was poorly absorbed whereas processed products such as tomato paste and lactolycopene had enhanced bioavailability[77].

Lycopene was also formulated into Lysosomal formulations and its potential implications on blood pressure and serum cholesterol levels were studied[10]. The Lysosomal formulation of Lycopene containing Dark Chocolate (DC) called L-tug, was found to produce a marked reduction in diastolic blood pressure and the levels of low-density lipoprotein (LDL) when compared against a standard group, administered with DC alone[78]. There was however only a small change in the systolic blood pressure with no significant reduction in the levels of HDL cholesterol, glucose, and C-reactive protein (CRP)[79].

Lycopene has also been formulated for delivery with a Nanostructured lipid carrier to enhance absorption after oral administration. Stability studies performed by storage at different time intervals for 90 days indicted no precipitate formation, no phase separation, and good dispersibility for the formulation[80]. Ex vivo gut permeation studies performed with the same also showed better results compared against the crude drug extract. The formulation was tested against breast cancer cells and their survival rates calculated using an MTT assay[81]. It was found that NLC loaded lycopene formulation demonstrated enhanced cytotoxicity against the crude formulation, attributing to the finding that, NLC loaded Lycopene formulation retained higher concentration within the cancer cells[82].

The nanodispersions form wherein the drug, which is a water-insoluble compound of interest, is dissolved in a volatile solvent system, that is finally evaporated at the end of the preparation process, has also been explored upon for Lycopene formulation[83]. Lycopene nanodispersions were successfully formulated by mixing an organic phase containing 0.3% w/v of lycopene powder dissolved in dichloromethane with an aqueous phase of 0.3% w/v T20 in deionized water at a ratio of 1:9 using the emulsification-evaporation method[84].

The Nanoparticle encapsulated formulations of Lycopene have been evaluated for in-vitro anti-cancer activity and inhibitory effect on skin inflammation and tumorigenesis in Swiss albino mice[85]. The active constituent was entrapped within a hydrophobic core surrounded by a hydrophilic outer shell. The nanoparticles were formulated using thermosensitive polymers to increase drug delivery. Microemulsion formulations of lycopene for topical delivery were found to enhance skin penetration and anti-oxidant activity. Lycopene has also been explored for vesicular nanocarrier formulations for dermal delivery[86].

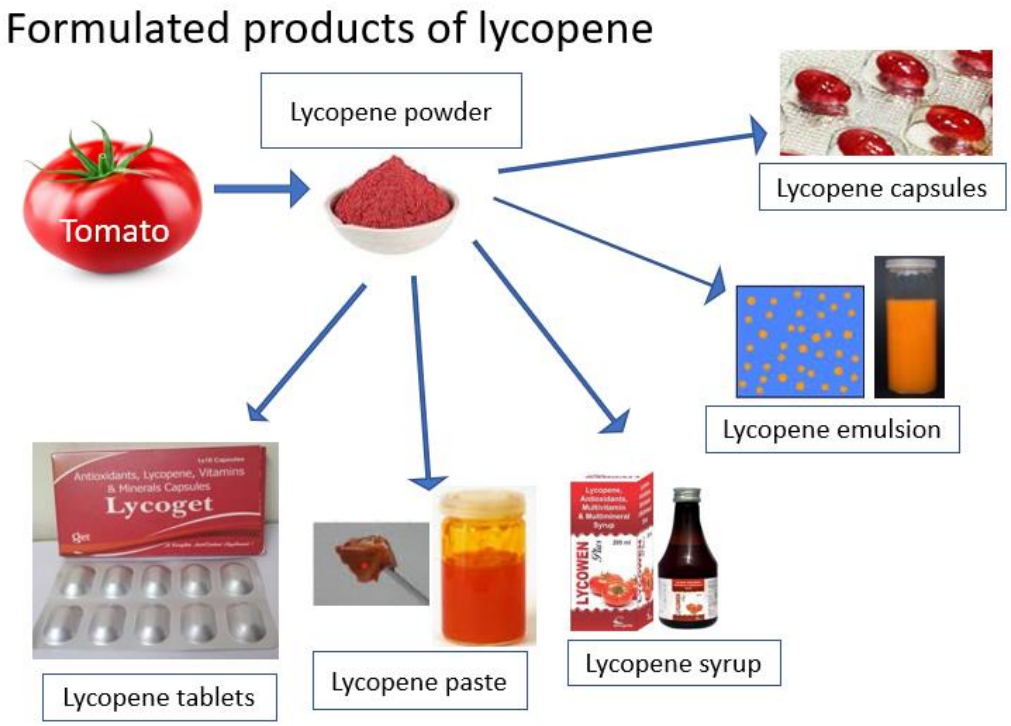

Fig:2. Various formulations of lycopene in the form of tablets, capsules, syrup, powder, paste and emulsions

VII. CONCLUSION AND FUTURE PERSPECTIVES

Over the years, the Chemopreventive and Cardioprotective activities of Lycopene have been extensively studied and well documented. The anti-cancer activities of lycopene have been implicated through various signalling pathways including apoptosis, cell cycle arrest, and the regulation of ROS-mediated cellular injury. Lycopene has also been found to act at the genomic levels by increasing the expression of pro-apoptotic molecules, p53, and decreasing the expression of anti-apoptotic molecules. The excellent oxygen quenching capacity or the antioxidant activity of lycopene has been found to play a central role in contributing to the health benefits of Lycopene. This review constitutes several novel formulations of Lycopene like nanodispersions, solid-lipid nanoparticles, and lysosomal delivery that yield promising data on bioavailability and absorption, in contrast to traditional formulations such as tablets and capsules. Such novel formulations could be used to enhance the therapeutic efficiency of Lycopene, by increasing absorption and bioavailability. However rigorous clinical trials must be conducted to validate such formulations and establish the optimal dose and toxicological data as higher concentrations of Lycopene have a negative influence on cardiomyocytes. With more trials on novel formulations of Lycopene, we could improve the potential use of Lycopene as a compound of nutraceutical and pharmaceutical significance.

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Conflict of interest

The authors declare no conflict of interest.

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