ISOLATION, PURIFICATION, CHARACTERIZATION AND ANTI-CARCINOGENIC ACTIVITY OF PROTEIN FROM AQUEOUS EXTRACT OF MEDICINAL PLANT PIPER LONGUM – A REVIEW

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ABSTRACT:

The present article is review of studies related to Isolation, Purification, Characterization and Anti-Carcinogenic activity of Protein from aqueous extract of medicinal plant Piper Longum. The curative properties of medicinal plants of long pepper are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The bio enhancing properties of piperine are being explored in a clinical trial in association with curcumin to assess whether the combination may reduce inflammation and discomfort from a ureteric stent in cancer patients

Key Words: *P. longum, anticancer potential, cancer cell.*

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INTRODUCTION:

An extensive review of the related literature on the problem similar to the study on Isolation, Purification, Characterization and Anti-Carcinogenic activity of Protein from aqueous extract of medicinal plant Piper Longum or related to it was done. It was done to get help in the planning of research. The study of related literature was done by finding, reading and evaluating reports of worldwide research.

Studies on Isolation, Purification, Characterization And Anti-Carcinogenic Activity Of Protein From Aqueous Extract Of Medicinal Plant Piper Longum is usually due to a certain factor which include socio-economic factors, lifestyle etc.

LITERATURE REVIEW:

The review of studies on Isolation, Purification, Characterization and Anti-Carcinogenic activity of Protein from aqueous extract of medicinal plant Piper Longum as a scientific critical of most important published scholarly literature is given here.

By increasing the bioavailability of other anti-tumorigenic spices and compounds, long pepper dramatically increases their potency and effectiveness against cancer. In addition long pepper directly counteracts cancer development, as piperine stops some of the pro-inflammatory cytokines that are produced by tumor cells. In the process, it interferes with the signaling mechanisms between cancer cells, thereby reducing the chances of tumor progression (Pradeep and Kuttan, 2020).

A report on evidence of carcinogenesis in mice shows that malignant tumours and multiple tumours were more in the pepper treated mice than untreated control. Pepper contains small amounts of safrole, a mildly carcinogenic compound. *In vitro* and *in vivo* studies revealed that piperine is efficacious in tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-based therapies in Triple negative breast cancer (TNBC) cells. Piperine inhibited the growth of human colon cancer cells and it is useful in in the treatment of colon cancer (Takao K *et al.,* 2020).

Piperine, the alkaloid from long pepper, contributes to the pungency, and volatile oil is responsible for the aroma and flavor. Piperine was first separated by Oersted (1820) as a yellow crystalline substance from the fruits of black pepper. Piperine is a weak base, which on hydrolysis with HNO3 or aqueous alkali yields a volatile base piperidine (C5H11N). The acidic product of hydrolysis is piperinic acid (C17H19O4). The structure of piperine is established as piperinic acid piperidide. Piperinic acid exists in four isomeric forms: 2-*trans,*4-*trans* (piperine); 2-*cis,*4-*trans* (isopiperine); 2-*trans,*4-*cis* (isochavicine) and 2-*cis,*4-*cis* (chavicine). Five analogues of piperine were separated and characterized by various workers. They are piperettine, piperanine, piperylin A, piperolein B and pipericine (de Paula VF et.al, 2020).

Piperine treatment of breast cancer cells showed reduced mitochondrial membrane integrity along with the release of cytochrome c and Smac/DIABLO from the mitochondrial cytosol. Furthermore, cytochrome c results in the formation of apoptosomes that result in the acceleration of the initiator caspase-9 Inhibitor apoptosis proteins (IAPs) are responsible for inhibition of caspase activity; however, Smac/ DIABLO induced by addition of piperine in breast cancer cells stops IAPs by binding to them and helps in acceleration of apoptosis of breast cancer cells as it presents the mechanism of piperine releasing mitochondrial cytochrome-c, which further activates apoptosome as well as inhibition of IAP by acceleration of Smac/DIABLO (Twiddy et al., 2019).

Long pepper is a powerful antioxidant by itself and also, enhances the action of other antioxidants. This makes black pepper particularly valuable in minimizing damages due to saturated fat rich diets, one of the main causes of oxidative stress. Long pepper maintains and enhances the levels and efficacy of important antioxidant compounds like glutathione, superoxide dismutase, catalase, glutathione peroxidase, vitamins C and E in vivo. In vitro studies have revealed that piperine can inhibit glutathione-Stransferase (s) (GST(s)) of parasite nematode (Azeez et al., 2019).

Expression of the MMP-2 and MMP-9 gene is necessary for induction of metastasis, as a high level of MMP-2 and MMP-9 proteins have been observed in breast tumor tissue (Köhrmann et al., 2009). They are also needed for the migration of TNBC. However, when TNBCs were treated with piperine MMP-2 and MMP-9, synthesis was inhibited. Action of piperine on inhibition of MMP-2 and MMP-9 that results in inhibition of breast cancer cell inhibition) However in contradiction to this, it was reported the potent cytotoxic effects of a piperine-free Piper longum extract opposite MCF-7 breast cancer cells and N-nitrosomethylurea-induced mammary tumorigenesis in rats (Venugopal et al., 2018).

Piperine, a main compound extracted from long pepper, has shown its anti-gastric cancer activity in several studies. Ling et al. have confirmed that IL-6 can induce gastric cancer cell invasion by activating the c-Src/RhoA/ROCK signaling pathway. Piperine was found to inhibit IL-6 expression, further suppressing cell invasion of gastric cancer cells (TMK-1). Earlier reports have shown that high levels of IL-6 are responsible for inducing gastric cancer cells. Furthermore, acceleration of the signal transducer and transcription-3 activator (STAT3) resulted in an increased level of IL-6 in gastric tumor cells (Sangwan et al., 2017)

Glutathione-S-transferase(s) (GST(s)) (E.C.2.5.1.18) are a large family of multifunctional dimeric enzymes that conjugate reduced glutathione to electrophilic centers in hydrophobic organic compounds. GST(s) are ample in mammals, plants and invertebrates. The components of the glutathione (GSH) system are main chemotherapeutic targets in filarial species, as it has been proposed to constitute the antioxidant system responsible for the long-term existence of filarial worms in mammalian host. Many inhibitors of enzymes involved in GSH metabolism are known to be associated with anti-parasitic activities because they inhibit the parasitic enzymes to a greater extent than the mammalian counterparts). GST provide the worm's primary defense against electrophilic and oxidative damage. The inhibition of parasite GST(s) thus deprives them of its main defense against oxidative stress and makes them unable to survive (Ramesh et al., 2017).

Lin et al. (2016) performed experiments with the action of piperine on human lung cancer cells (A549) and on human lung fibroblasts (W138) and their findings revealed that piperine can inhibit cell proliferation and can also induce apoptosis in A549. However, piperine did not show toxicity against human lung fibroblasts (W138). Piperine from long pepper was found to express a high level of the p53 gene in a concentration manner. Thus, a high level of p53 expressed in the presence of piperine could induce cell cycle arrest in the G2/M phase in A549 cells (Lin et al., 2016).

Two active principles, ethyl-3',4',5'-trimethoxycinnamate and piperine separated from the combined hexane and chloroform extracts of P. longum significantly blocked the adhesion of neutrophils to endothelium in a time and concentration dependent manner. Piplartine (piperlongumine) and piperine alkaloidal amides separated from Piper showed cytotoxic activity towards several tumor cell lines. It was demonstrated that the medicinal properties of piperine - antioxidant, anti-apoptotic, and restorative ability against cell proliferative, mutagenic response and phenotypic alterations - suggests its therapeutic usefulness in immune compromised conditions (Samykutty et al.; 2016).

Orally administered aqueous suspension of P. longum root powder had weak opioid but potent nonsteroidal anti-inflammatory drugs (NSAID) type of analgesic activity. The fruit decoction showed anti-inflammatory activity against carrageenin induced rat paw edema. The immunoregulatory potential of P. longum and piperinic acid, one of its active constituents, in mice and human peripheral blood mononuclear cells models showed a dose dependent decrease of lymphocytes (CD4+ and CD8+ T cells) and cytokine levels. Aqueous extract of P. longum fruit powder showed 100% giardicidal activity. A popular Ayurvedic preparation containing long pepper, 'Pippli rasayana' was tested in mice infected with Giardia lamblia and found to produce significant acceleration of macrophages, as shown by an increased macrophage migration index and phagocytic activity (Hegeto et al., 2016).

Primary metabolites, such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, are found in all plants and perform metabolic - structural and functional - roles that are essential and usually evident. Secondary metabolites, on the other hand, are a vast and diverse assortment of organic compounds of considerable complexity of chemical structures and biosynthetic pathways, the great mainity of which do not appear to participate directly in growth and development, and are differentially distributed among limited taxonomic groups within the plant kingdom. Their functions, many of which have hitherto been unknown, are being elucidated with increasing frequency, like adaptation of plants to their environment, a source for drug lead compounds), antimicrobial effects. Furthermore, evolution has already carried out a screening process whereby plants are more likely to survive if they contain potent compounds which deter animals from eating those (Rey-Ladino et al., 2015).

Pharmacological inhibition of acylCoA:diacylglycerol acyl transferase by alkamides from the fruit extracts of P. longum was a potential therapy for the treatment of obesity and type 2 diabetes. Guineensine, separated from the chloroform extract, inhibited acylCoA:Cholesterol acyl transferase (ACAT) activity in a dose-dependent manner . Dehydropipernonaline, an amide from the fruits of P. longum was reported to have coronary vasorelaxant activity Four acidamides from the fruits of P. longum, piperine, pipernonaline, piperoctadecalidine and piperlongumine, especially the last, showed dosedependent inhibitory activities on rabbit platelet aggregation induced by collagen, arachidonic acid, and platelet-activating factor, except for that induced by thrombin. Curcuminoids-piperine combination is an efficacious adjunctive therapy for metabolic syndrome treatment and it can modify serum lipid concentrations (Sharma et al., 2015).

The benzene extract of P. longum in combination with methanol extract of Embelia ribes berries inhibited pregnancy in 80% of animals. Piperine showed marked increase in serum gonadotropins and a decrease in intra-testicular testosterone concentration, despite normal serum testosterone titre. The crude extract of P. longum and its hexane fraction exhibited 100 and 86% efficacy respectively in female rats The reproductive toxicity of piperine in Swiss albino mice was through increased period of the diestrous phase, which seemed to result in decreased mating performance and fertility; thus piperine interferes with several crucial reproductive events in a mammalian model (Lin et al., 2014).

Various extracts of P. longum exhibited better antibacterial activity against bacterial pathogens such as Streptomyces albus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli and Bacillus megaterium and the fungus, Aspergillus niger, than streptomycin. The separated constituents and n-hexane extract showed varying degrees of antibacterial activity against all the tested bacteria. The essential oil from fruits and whole plant extracts of long pepper exhibited fungicidal activity on the phytopathogenic fungi Pyricularia oryzae, Rhizoctonia solani, Botrytis cineria, Phytophthora infestans, Puccinia recondita and Erysiphe graminis. A piperidine alkaloid, pipernonaline, separated from the hexane fraction of P. longum showed potent fungicidal activity against P. recondita of 91% and 80% at concentrations of 0.5 and 0.25 mg ml−1, respectively (Kokate et al., 2013)

The reported experiments constitute a part of our ongoing ef-forts directed towards translating Ayurvedic therapeutic principlesin terms of molecular concepts of modern medicine and to obtaintherapeutic leads from conventional ly known therapeutic plantspotentially useful for prevention and cure of psychosomatic disor-ders commonly associated with systemic inflammation.29In this study, a pharmacologically well characterized mouse bioassay procedure evolving from our efforts to estimate therapeutically interesting doses and dosing regimen of stress response regulating therapeutic plants and their bioactive constituents30e32was used for comparing the efficacies of doxycycline like adaptogenic efficaciesof piperlongumine and a commercially available P. longum extractin mice. In a further experiment, aspirin like analgesic efficacies oftheir stress response suppressing doses and treatment regimenwere compared in a slightly modified version of the bioassay procedures used in the pilot dose finding experiments (Elnaggar *et al*., 2012)

Piperlongumine and piperine are structurally analogous bioac-tive molecules and both of them have been reported to possessantimicrobial activities. It is now becoming increasingly apparentthat gut microbiota play a crucial role in regulating physiologicalstress responses,21and that depending on their doses and treat-ment regimen used, bactericidal and other agents with modulatingeffects on gut microbial ecology can have diverse health bene-fits. For example, appropriate doses and treatment regimen of doxycycline like antibiotics can suppress physiological stress response and can reset the neuro-hormonal status regulated by gutmicrobiota (Pachauri *et al.,* 2010).

Trikatu is one such herbal mixture containing equal parts of P. longum and P. nigrum fruits and Zingiber officinalis root powder. This mixture is often used in many Ayurvedic formulations commonly prescribed for treatments of gastric and abdominal disorders, asthma, bronchitis, coughs, dysentery, pyrexia, insomnia, colic and intestinal infection. Diverse stress response regulating potentials of all the three-plant used in this mixture have been reported, and such properties of P. longum extracts and their analgesic, ulcer protecting and diverse other therapeutically interesting bioactivities of piperine in animal models are also well known. However, as yet no reports on the role of piperlongumine in stress response modulating, pain relieving, and diverse other conventional ly known medicinal uses of extracts P. longum and other plants of Piperaceae family have appeared (Bhalekar *et al.,* 2010)

The fruits of P. longum were identified and the accession number 006 was obtained. The phytochemical analysis revealed the presence of tannins, flavonoids, glycosides, phenolics, diterpenes and triterpenes in extract and fractions of P. longum. The extract and fractions were diluted serially in 6.25 per cent tween 80 to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 mg/mL. Ivermectin and thiabendazole at 10 µg/mL acted as positive controls and 6.25 per cent tween 80 as negative control. The methanolic extract was highly active against ova with IC50 of 0.026 mg/mL. The n-hexane fraction was potent in inducing larval mortality with IC50 of 1.383 mg/mL while chloroform fraction inhibited larval migration with IC50 of 1.796 mg/mL (Shaikh *et al.,* 2009)

Amphistomes were highly sensitive for methanolic extract of P. longum which possessed IC50 of 5.493 mg/mL Based on IC50 values, the methanolic extract was found to be most potent while chloroform fraction was effective against ova, larvae and also adults. GCMS analysis of potent methanolic extract revealed the presence of piperidinone, hydrocinnamic acid, ethylhexahydro azepine, methyleugenol, hexadecanoic acid and caryophyllene oxide which may have contributed for the anthelmintic activity. The acute oral toxicity study revealed mild vascular changes in liver. From the present study, it can be concluded that chloroform fraction of P. longum possessed maximum broad spectrum anthelmintic activity comparable to controls (Moorthi *et al.,* 2009)

DPPH radical scavenging activity was assessed according to the method of Shimada et al. (1992) with minor modifications. The Piper longum extracts at a concentration of 25 µg was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37°C for 30 minutes and measured spectrophotometrically at 517 nm. Ascorbic acid, BHA and Curcumin were used as positive control under the same assay conditions. Negative control was without any inhibitor or Piper longum extracts. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The

%DPPH radical scavenging activity of different extracts of Piper longum was calculated from the decrease in absorbance at 517 nm in comparison with negative control (Abraham and Platts, 2008)

The Superoxide radical (O2•-) scavenging activity of spice Piper longum extracts was measured according to the method of Lee et al. (Lee et al., 2002) with minor modifications. The reaction mixture containing 100µl of 30mM EDTA (pH 7.4), 10µl of 30mM hypoxanthine in 50mM NaOH and 200µl of 1.42mM nitro blue tetrazolium with or without Piper longum extracts and SOD serving as positive control at various concentrations ranging from 50-300µg. After the solution was pre-incubated at ambient temperature for 3min, 100µl of Xanthine oxidase solution (0.5U/ml) was added to the mixture and incubated for one hour at 37C, and the volume was made up to 3ml with 20mM phosphate buffer (pH 7.4). The solution was incubated at room temperature for 20 min; absorbance was measured at 560 nm. Appropriate controls were included to rule out the artifacts induced reaction. The control was without any inhibitor (Goel *et al.* (2007).

The superoxide radical scavenging activity of Piper longum showed at 15g dosage, the aqueous extract showed more Superoxide radical scavenging activity (28%) when compared to methanol (23%), petroleum ether (14%) and acetone (16%) extract, and to the standard antioxidants like Ascorbic acid (43%), and alpha-tocopherol (52%). These studies are encouraging us to do further studies towards purification of the aqueous extract of Piper longum (Shoba *et al*., 2007).

The aqueous extract of Piper longum have showed more significant antioxidant activity due to bioactive components like protein, total phenols, flavonoids and are subjected to pharmaceutical drug formulations. This plant is inexpensive, readily available, and effective for treating many diseases, including cancer, inflammation, depression, diabetes, obesity, and hepatotoxicity. In this study the protein concentration in aqueous extract of Piper longum is considerably higher than solvent extracts (Gyapong *et al.,* 2005).

Different seeds' ethanolic extracts (50, 70, or 100% ethanol) were studied in three colorectal cell lines. The highest cytotoxic effect was seen for the 50% seeds' ethanolic extract (EEPN). The highest biological activity of EEPN was imputable to the highest content of total phenolic compounds extracted. Additionally, EEPN showed antioxidant and anti-inflammatory properties, which were assessed by biochemical assays. No insight into the molecular mechanisms of EEPN's cytotoxicity was provided. Recently, it was investigated the anticancer activity of a Piper longum water seeds' extract formulated as SnO2 nanoparticles in colorectal (HCT-116) and lung (A549) cancer cell lines. They demonstrated that higher dose and smaller size nanoparticles generated more reactive oxygen species (ROS) and hence exhibited a higher cytotoxicity compared to larger size nanoparticles, underlying the crucial role of formulation in improving the biological activity of Piper longum preparations (Jafri *et al.,2005)*.

The anticancer activity of a macerated ethanolic extract of Piper longum fruits was explored in both in vitro and in vivo breast cancer models. Treatment with the extract induced intracellular oxidative stress, which was considered the main component responsible for its cytotoxic effects in cancer cells. Since ROS can cause DNA damage, the observed oxidative DNA damage corroborated ROS involvement in the anticancer effects of the extract. These findings were confirmed in vivo, where increased lipid peroxidation and protein carbonylation and an elevated activity of the antioxidant enzymes were recorded. The same research group investigated the anticancer potential of a high-pressure extract from unripe fruits of the black pepper cultivar Bragantina, obtained by supercritical fluid extraction (SFE). SFE represents an energy-efficient and environmentally friendly extraction technology that helps to overcome the limitation of the poor solubility of molecules such as piperine. The SFE extract showed a higher content of piperine and the highest cytotoxic activity compared to conventional ethanolic extracts (Siddiqui *et al.,*2005).

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