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NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *CURCUMA LONGA* AGAINST CISPLATIN NEPHROTOXICITY

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ABSTRACT

Cisplatin is a potent antitumor agent, but its clinical use is limited due to its renal toxicity. Several pharmacological studies have reported beneficial effects of certain Indian Medicinal plants to protect from kidney and renal injuries. In the present investigation, the ethanolic extract of dried *curcuma longa* was evaluated for nephroprotective activity in Cisplatin induced renal damage in rats. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of Cisplatin 5mg/kg. Effect of concurrent administration of *curcuma longa* ethanolic extract at a dose of 250mg/kg given by oral route was determined using serum creatinine and blood urea and change in body weight as indicators of kidney damage. Cystone was used as standard drug. The extract significantly decreased the cisplatin induced nephrotoxicity. Kidney plays a prominent role in the metabolism and excretion of many exogenously administered drugs, diagnostic agents and their metabolites. Nephrotoxins are drugs or chemicals that produce toxic effect on kidney Nephrotoxicity is one of the major side effects of cisplatin. Several studies have shown that cisplatin induces renal damage by free radical generation. Remarkable changes were observed in body weight, serum creatinine and urea levels. It was observed that the ethanolic extract significantly protected the kidneys from injury. Current study results show that the ethanolic extract of *curcuma longa* is an excellent nephroprotective as compared to cystone.

KEYWORDS

Curcuma longa, Nephroprotective activity, Cisplatin, Cystone, Nephrotoxicity, Erythropoietin, CDDP (Cis-Diamine Dichloro Platinum) and Curcumin (Diferuloylmethane).

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INTRODUCTON

Kidney disease is of epidemic proportions and its prevalence will, double in the next twenty- five years, particularly in the developing countries[1]. There are now over one million dialysis patients worldwide with an incidence of about 500,000 new patients each year[2-4]. When the kidneys are exposed

to toxic agents, either accidentally or intentionally, alteration in morphology may occur which will directly affect the glomerulus and renal tubules and subsequently result to kidney problem[5]. According to World Kidney Day[1], the major causes of kidney disease include diabetes, high blood pressure, urinary tract infection, obesity, glomerulonephritis and polycystic with diabetes accounting for nearly 44%. Furthermore, World Health Organization[3] statistics revealed that more than 36.8 million Nigerians are suffering from various forms of kidney disease which suggest that one in ten Nigerians is suffering from some form of kidney disorder or another with the majority of them die every day due to poverty, ignorance, cost and inaccessibility to treatment. It has been revealed that, kidney damage and kidney related diseases cause more death than malaria and HIV/AIDS in Nigeria.

Adult human kidney weighs about 160g, 11.25cm long, 2.5cm thick and 5.5-7.7cm wide. This retroperitoneal organ are paired, bean shaped, reddish brown, located at the level of the twelfth thoracic and third lumbar vertebrae on either sides of the vertebral column. It is protected by three layers of tissue. These layers consists of (1) renal capsule, an innermost strong fibrous layer which protects kidney from shock and infections (2) the adipose capsule, middle layer of fatty tissue which keeps kidney in right position (3) the renal fascia, outermost fibrous connective tissue which bind kidney with the abdominal wall. Cut surface of a bisected kidney shows two anatomically distinct regions: the pale outer region called renal cortex and the inner dark region knownas renal medulla[4,5].

Nephrotoxicity

Kidney plays a prominent role in the metabolism and excretion of many exogenously administered drugs, diagnostic agents and their metabolites. Nephrotoxins are drugs or chemicals that produce toxic effect on kidney[6,7]. Through renal arteries nephrotoxins reach the functional units of kidney known as nephrons. Hence cells of nephrons are more susceptible to drug related toxic responses as it is exposed to high concentration of drug and metabolites. Previous research studies have reported that the acute kidney injury have been a serious adverse effect of various drugs, compounds used in industries and diagnostic agents like radio contrast media[8].

Kidney injury associated with certain drug administration may cause either cumulative dose-dependent toxicity or idiosyncratic dose-independent toxicity. The morphological examination of the kidney biopsy due to nephrotoxic insult exhibited alterations in the normal structures of the nephron, indicating damage to the renal infrastructure. Drug induced renal failure can be further classified based upon the renal compartments principally affected with toxicity as acute tubular necrosis, interstitial nephritis, glomerulonephritis, renal vascular damage and intrarenal obstruction. Acute tubular necrosis is the most common form of intrarenal failure associated with prolonged exposure of kidney to nephrotoxin or due to ischaemia, sepsis, diabetes and artherosclerosis[9]. Interstitial nephritis is related with nephrotoxin-induced hypersensitivity reaction or inflammation affecting interstitium. Common cause of glomerular nephritis is inflammatory response due to the deposition of immune complexes (exogenous or endogenous antigens) in glomerulus[11]. Damage to the visceral layer of the Bowman's capsule-podocytes may cause a disorder known as nephritic syndrome, which further leads to glomerulonephritis. Occlusion of renal artery or vein also causes alterations in intrarenal vasculature. Deposition of debris provoke intrinsic renal damage.

Epidemiology of drug induced nephrotoxicity

Drug-induced acute kidney injury accounted for 20% of all acute renal failure cases in an Indian study[14]. Prospective cohort studies have reported that the incidence of drug related kidney toxicity is about 14-16% of adult population. Nephrotoxicity linked with the administration of aminoglycoside antibiotics elevated from 3% in 1969 to 10-20% in 2010[8,12]. Another study reported that 27 million persons are suffering from chronic kidney disease. Drastic increase in drug related nephrotoxicity (about 30%) has been observed over the last 10 years[13]. Drug related renal injury is a common

condition present even in children and estimated about 16% of hospitalized acute renal failure events[14]. Incidence and prevalence of chronic kidney disease and end-stage renal disease is dramatically increasing globally[15]. Kidney stones is a painful disorder and still it remain as common worldwide problem. Recurrent rate is high, more than 50% in 5-10 years. It is accounted that 12% of the world population is affected by kidney stones and male/female ratio is 3:1. Relapse rate is more in male (70 to 80%) than in females (47 to 60%).

Etiology

Various factors are involved which enhance the drug induced nephrotoxicty. An easiest approach to know about the vulnerability of kidney nephrotoxins entails the classification of risk factors into three major categories as patient-specific, kidney- related and drug-related factors. Usually, more than one risk factors are involved in drug related renal injury affecting all renal compartments and cause one or more renal syndromes.

Cisplatin-induced nephrotoxicity

Cisplatin is chemically known as cis diamine dichloro platinum (II), CDDP. It is the most potent, highly effective and currently available alkylating agent used in the treatment of solid tumours like breast cancer, head cancer, neck cancer, testes and ovarian cancers etc[17]. However, clinical utilization of this heavy metal complex is restricted due to the adverse reactions including ototoxicity, gastrotoxicity, myelosuppression, allergic reaction and nephrotoxicity[18]. Cisplatin induced nephrotoxicity occurs in about 20-30% of patients[19]. Factors which promote cisplatin induced nephrotoxicity include high perfusion rate, drug uptake of free-form cisplatin in the proximal kidney tubule, metabolism and disproportionate accumulation of platinum concentrations in the kidneys greater than other organs[20]. Major clinical manifestations of cisplatin induced nephrotoxicity include erythropoietin deficiency, proteinuria, renal salt wasting, hyperuricemia, renal concentrating defect, hypocalcemia, hypomagnesemia and hypokalemia.

Through glomeruli cisplatin enter the tubular lumen of the nephron either by passive process or facilitated transport mechanism. Cisplatin administration may precipitate acute renal failure as it causes vasoconstriction of afferent arterioles, leading to the decreased blood flow to the Bowman's capsule. Cisplatin stimulates various signaling pathways (mitogen-activated protein kinase, tumor protein p53, reactive oxygen species) of the tubular cells and induces tumor necrosis factor-alpha formation which leads to inflammatory response, tubular cell injury or necrosis. The intrinsic mitochondrial and extrinsic death receptor may also gets activated which further leads to caspase-dependent or independent apoptosis. Inequilibrium between cyclin-dependent kinase (CDK2) or cyclin-dependent kinase inhibitor proteins (p21) is the major causative factor which induce cell apoptosis and acute renal failure. Cisplatin administration causes induction of inhibitory protein p21 and the activation of CDK2 protein which results in renal tubular cell toxicity. DNA injury stimulate ATR (ataxia telangiectasia and Rad3-related protein) which leads to stimulation of inhibitory protein p53 and phosphorylation. This inhibitory protein further causes transcription of p53 upregulated modulator of *apoptosis (*PUMA) and p53-inducible death-domain-containing protein, (PIDD, apoptosis genes) in the neuron cells. PIDD genes promote caspase-2 activation and secretion of apoptosis-inducing factor (AIF) from the mitochondria of the tubular cells and causes caspase-independent apoptosis. The PUMA-alpha translocates to the cellular power house, interacts with cellular components, neutralizes anti-apoptotic.

Importance of herbs in nephroprotection

Plant drugs have been used for treating numerous health problems all over the world as it is generally considered to be free from side effects due to their natural origin. The trend of using herbal products has increased and the active plant extracts are frequently screened for new drug discoveries. It has been observed that modern medicine used for the treatment of many diseases like kidney diseases, liver disorders, cardiovascular diseases, arthritis, asthma and skin diseases associated with serious adverse effects. Hence in recent years numerous

research works have been carried out on medicinal plants for their claimed activities.

Several research studies confirmed that the generation of free radicals and diminished antioxidant activity are implicated in development of several life limiting chronic diseases and xenobiotics-induced nephrotoxicity. Inhibition of protein synthesis, DNA damage, mitochondrial injury, apoptotic cell death are the various mechanism associated with oxidative stress in drug induced nephrotoxicty.

Prolonged exposure of kidney to drugs (gentamicin, cisplatin, NSAIDs and cyclosporine), chemical reagents (ethylene glycol, carbon tetra chloride, sodium oxalate) and heavy metals (lead, mercury, arsenic and cadmium) lead to renal injury. Nephrotoxicity is a multifactorial process linked with various etiological factors, hence the treatment is aimed at multiple targets. Medicinal plants have curative properties on nephrotoxicity due to the presence of various chemical constituents aiming at multiple targets and may offer effective, inexpensive and safe remedy. Early literatures have prescribed numerous herbal drugs for the treatment of renal disorders and also reported that concurrent administration along with different nephrotoxic agents reduced the toxic effects. Many herbs have been proven to be effective as nephroprotective agents while many more are claimed to be nephroprotective but there is lack of scientific evidence to support such claims.

PLANT PROFILE

Introduction

Curcuma longa L. is an herbaceous perennial plant, belonging to the family *Zingiberaceae*. It has a large oval rhizome with sessile cylindrical tubers, orange coloured inside. Its leaves start from the rhizome, are elliptical and can reach up to 1.2 m in length. Its flowers are yellow, between 10 to 15 cm in length and they group together in dense spikes, which appear from the end of spring until the middle of summer. No fruits are known for this plant. The Curcuma genus contains around 30 species. The plant originates from India and South-East Asia. In India, it is popularly known as "Haldi". It has been well studied in Malaysia, Indonesia and India due to its economic importance. The rhizomes of turmeric are commonly used as a flavoring, coloring agent and preservative. Commercially, it is traded as a dye, spice and source of industrial starch[1].

CHEMICAL CONSTITUENTS

The principle constituent of turmeric is Curcumin, which is diferuloyl methane. Other constituents are curcuminoids and an essential oil called zingiberine. Its chemical study shows that it contains proteins, carbohydrates and fibre. Its mineral and vitamin contents are calcium, phosphorus, iron, carotene, thiamine and niacin. It contains 5% of volatile oil, resin, abundant Zingiberaceous starch grains and yellow coloring substances known as curcminoids. The components of curcuminoids are known as curcumin (50-60%). Chemically, curcuma species contain volatile oil, starch and curcumin. Curcumin and other related curcuminoids are reported to be responsible for the yellow colour in some species. Volatile oil content ranges from 1 to 6.5% and composed of mono and sesquiterpenes such as alpha and beta -pinene, alpha- phellandrene, camphene, camphor, zingibereneand alpha, beta curcumenes. Species like C. angustifolia and C. caulina have high starch content and are used as a substituted for arrow root. Chemical constituents are known to vary as per geographical locations and curcumin is reported to vary from 1 to 10%[21].

CHEMISTRY

The major constituent, curcumin (diferuloylmethane) is in the most important fraction of *Curcuma longa* L. and its chemical structure was determined[12]. It melts at 176-177°C and forms red-brown salts with alkalis. Curcumin is soluble in ethanol, alkalis, ketone, acetic acid and chloroform; and is insoluble in water. In the molecule of curcumin, the main chain is aliphatic, unsaturated and the aryl group can be substituted or not. Curcuminoids are between 2 and 9%. Their main components are: curcumin (60%), desmethoxycurcumin, monodemethoxycurcumin, bisdemethoxycurcumin, dihydrocurcumin and

cyclocurcumin. Curcumins oxidation yields vanillin.

USES

Curcumin is the part of turmeric that gives curry food its golden color. This also provides turmeric with curcuminoids, which are believed to have health properties such as antioxidant, antibacterial and anti- inflammatory qualities. Turmeric benefits have been known for centuries and have always been an important part of Chinese herbal medicine and also the Ayurvedic medicine of India. This natural food is believed to support liver health, help prevent bad cholesterol and it is being studied for its ability to block tumors. A strong antioxidant, turmeric is rich with a substance believed to protect body cells from damage caused by oxidation. Oxygen free radicals may suppress immune function and cause t issue damage. In addition to their anticancer effects, antioxidants in turmeric protect the brain, kidneys, and liver from damage by alcohol, drugs, radiation, heavy metals or chemicals. For skin problems, turmeric ointment, or a paste made from powdered turmeric is applied directly to the skin, as often as needed. It's used to treat cuts, scrapes, and skin conditions such as acne, diaper rash and psoriasis. Turmeric essential oil is also used in eczema and eliminates spots. Turmeric is used as carminative, which means it is used as a tonic to remove gases from the stomach that causes stomach upset[14].

PROPERTIES OF TURMERIC

Anti-inflammatory activity

There are a great number of compounds extracted from *Curcuma Longa* L. being potent inhibitors of inflammation.

Anti-inflammatory activity of curcumin and other semi-synthetic analogues (sodium curcuminate, diacetyl curcumin, triethyl curcumin and tetrahydro curcumin) in carrageenan- induced rat paw edema and cotton pellet granuloma models of inflammation in rats was reported[15]. Curcumin and its analogues showed similar action in carrageeninin induced paw edema in rats; however the sodium curcuminate was the most potent analogue and was more water-soluble than curcumin. Among the curcumin analogues, triethyl curcumin was the most potent anti-inflammatory in the chronic model of inflammation, when compared with the others and with the drug reference; and tetrahydro curcumin showed no activity.

In the acute inflammation condition, all the substances were more effective. Activity of the compounds used in these experiments, would depend on the model of inflammation.

The anti-inflammatory activity in different fractions of the petroleum ether extract of the rhizomes of turmeric (two constituents) in animals was investigated[22]. Inflammation is the starting point in the skin ageing process. An inflamed area is in reality, a micro -wound, which, stimulated by certain environmental factors (ultra-violet rays, contamination, etc.), progresses to a wrinkle or skin imperfection. The inflammation also affects the skin pigmentation.

Mode of action

Curcumin showed anti-inflammatory properties in animal models by inhibiting the activity of the enzymes cycloxygenase-2 and lipoxygenase as well as the enzyme nitric oxide synthase[13].

ANTIOXIDANT ACTIVITY

It is known that the damages caused by oxidation in the different cellular components are one of the main causes of many diseases, including ageing[16].

Mode of action

Curcumin has a free radical scavenger activity, especially on the hydroxyl radical, which explains its capacity to protect DNA from damage in human cell cultures exposed to radiation. *In vitro* studies have demonstrated its capacity to block the activity of the cyclooxygenase and lipooxygenase enzymes. Topical applications of curcumin on the skin of mice increase the glutathione level and the glutathione Stransferase activity, while at the same time, inhibits lipid peroxidation in the skin tissue. The local application of turmeric extract has a recognised antioxidant and anti- inflammatory activity. It is more efficient than vitamin E as an anti-radical agent and as an inhibitor of lipid peroxidation[23]. Local applications of curcumin may

noticeably inhibit the ODC (ornithine decarboxylase) activity induced by simultaneous UVA radiation and TPA (tetradecanoylphorbol acetate) application on mouse epidermis. It is accepted that such activity of curcumin may be due to its capacity to scavenge free radicals or to interrupt the activation of protein kinase C[24]. In rats with ethanol-induced brain damage, curcumin exerted protective effects, which were mainly due to its antioxidant activity resulting from the increased glutathione levels and decreased lipid peroxidation in neuronal membranes. Additionally, certain curcuminoids are present in the rhizome. Curcumin, demethoxycurcumin and bisdemethoxycurcumin protected *in vitro* human umbilical cord endothelial cells and rat pheochromocytome cells against the entry of beta-amyloidal, a substance that induces oxidative stress and is involved in the neuronal deterioration observed in Alzheimer. It was demonstrated that the water and ethanol extracts of turmeric rhizomes inhibit the oxidation of erythrocyte membranes and hepatic microsomes of rabbits undergoing an atherogenic diet[13].

Tetrahydrocurcumin, a hydrogenated derivative has been isolated from curcumin[16]. This molecule has a strong antioxidant action because its structure includes a phenol group and a β-diketone. The relationship between molecular structure and activity of tetrahydrocurcuminoids has also been reported[23]. Thus, the antioxidant activity of turmeric extract makes it highly recommendable when formulating cosmetic products destined to protect the skin and hair from oxidative processes. The antioxidant activities of curcumin and related compounds have been investigated by a variety of assay systems, in both *in vitro* and *in vivo* conditions. The disparity in assay conditions makes exact comparisons rather difficult.

ANTIMICROBIAL ACTIVITY

It has been shown that curcumin *in vitro* is highly toxic to Salmonella sp. but not to *Escherichia coli*.

Mode of action

The water and ethanol extracts of turmeric rhizome have a moderate inhibitory activity on Staphylococcus sp. and Escherichia coli. Other *in vitro* studies evidenced that the essential oil has a weak inhibitory activity on *Staphylococcus aureus, S.epidermidis, Proteus vulgaris* and *Aspergillus fumigatus*. As anti-protozoa agents, curcumin and bisdemethoxycurcumin showed moderate *in vitro* activity against Plasmodium falciparum and Leshmania major. The water and ether extracts of turmeric showed repellent effects on the insect species *Aedes aegypti, Rhizopertha dominica, Sitophilus oryzae, Spodeptera litura and Tribolium castaneum, antifungal effects against Helminthosporium sp., Pyricularia oryzae, Rhizoctonia solani, Sclerotium oryzae and Sclerotium rolfsii* and nematicide effects against *Meloidogyne incognita*. Turmeric essential oil showed repellent effects on the mosquitoes *Aedes aegypti, Anopheles dirus* and *Culex quinquefasciatus*. Such repellent activity is strengthened by the addition of 5% vanillin. The hexane extract of turmeric rhizome inhibited the growth of the fungi Piedraia hortae, Trichophyton mentagrophytes and Microsporum cannis. It also inhibited Aspergillus sp. A preliminary study carried out in India with 814 patients with scabies demonstrated the efficacy of local applications of a turmeric rhizome paste, which did not produce toxic or adverse effects[13]. The anti-bacterial *in vitro* activity of the turmeric alcohol extract, curcumin and its essential oils against Gram-positive bacteria is well known. Significant anti-fungal activity has also been described. Turmeric essential oils have demonstrated anti-fungal activity on being applied topically on guinea pigs and in vitro tests against different isolated pathogens[25]. Thus, the antimicrobial action of turmeric extract makes it a very recommendable component when formulating cosmetic products with antiseptic activity as well as cosmetic products with an insect repellent function.

ANTICANCER AND ANTICARCINOGENIC ACTIVITY

It was recognized that anticarcinogenic properties of classical Michael acceptors[26] have been demonstrated in curcumin[27] and it has been suggested that the presence of a hydroxyphenyl

group in compounds analogous to curcumin, especially in the 2-position, is supportive of the chemoprotective activity through the ability to induce Phase II detoxification enzymes.

Mode of action

The inhibition of formation of the Fos-Jun-DNA complex, the presence of a 4- hydroxyphenyl, flanked by an adjacent methoxy or nitro group on the phenyl ring in curcumin analogues, conferred better potency than curcumin[27].

Showed that the monosemicarbazone of curcumin has greater cytotoxic activity than curcumin itself. In one of the more significant findings on the anticancer activity of compounds inspired by curcumin[28,29] announced the superior activity of 2, 6-bis (2-fluorobenzylidene) piperidon in antiangiogenesis, cell cycle arrest and apoptosis of cancer cells. These authors observed that the bis-benzylidene piperidone, pyrone, and cyclohexanone derivatives, containing α, β-unsaturated ketone unit, exhibit much greater anticancer and antiangiogenesis activities than curcumin, with its 1, 3- diketone unit.

ANTIVENOM ACTIVITY

The fraction consisting of ar-turmerone, isolated from *Curcuma Longa* L, neutralized both the hemorrhagic activity and lethal effect of venom in mice. In this study ar-turmerone was capable of abolishing the hemorrhagic activity of Bottrop's venom and about 70% of the lethal effect of Crotalus venom. Ar-turmerone can act as an enzymatic inhibitor in the case of venom enzymes, with proteolytic and hemorrhagic activities[30].

ANTI-HIV ACTIVITY[31]

Demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor and suggested that curcumin analogs may be developed as anti-AIDS drugs. Curcumin inhibited the replication of HIV-1 integrase protein[2]. Reported that curcumin was claimed for anti-HIV-1 and HIV-2 activities in a recent patent application.

WOUND HEALING ACTIVITY

The topical administration of curcumin extracts on skin wounds on the skin of diabetic rats demonstrated an improvement in the wound healing process.

Mode of action

The reparation action mechanism involved an increase in the levels of beta transforming growth factor plus an increase in the activity of the enzyme nitric oxide synthase[13]. The wound-healing activity of turmeric has been widely studied and it has been seen that its local application is effective. In Chinese medicine it has been used for this purpose since ancient times[25]. This action makes turmeric extract a good ingredient when formulating cosmetics with regeneration activity.

PHOTO-PROTECTOR ACTIVITY

Mode of action

The action is due to its antioxidant activity, 25% of the lipids of the surface of the skin are unsaturated, and therefore, are easily attacked by free radicals. The ultraviolet rays of the sun penetrate the skin and accelerate the damage caused by these radicals. Prolonged exposure to these radiations means that the collagen and elastin fibres, responsible for the elasticity and integrity of the skin, may be degraded by inherent enzymes, thus causing deterioration in the texture of the skin. In laboratory studies, extract of turmeric was shown to be effective in suppressing inflammation and protecting the epidermal cells from the damages caused by ultraviolet B radiation[23]. Curcumin in small doses has been shown to have the capacity to protect against chromosomal damage caused by gamma radiation. Curcumin has also been shown to inhibit the mutagenic induction effect of UV rays[13].

MATERIAL AND METHODS

Materials

Plant material collection

Powder of *Curcuma longa* was collected in March 13th 2023, The *Curcuma longa* was authenticated by a Botanist Dr.S.L. Mourya.) Sr scientist, Jawaharlal Nehru krishi vishwvidyalaya. After collection, the *Curcuma longa* was washed with distilled water and

air dried at room temperature and then powdered with the aid of surface sterilize pestle and mortar and passed through a sterile sieve to obtain the required particles of uniform size.

Plant extraction and fractionation

Powder of *Curcuma longa* (10) was macerated in 500ml (90% v/v) ethanol and left in air tight aspirator bottle for 72 hours with occasional stirring with a sterilized glass rod to ensure efficient extraction. The extract was then separated from the sample residue by filtration through What man No.1 filter paper (Alade and Irobi[13] as modified by Karumi *et al*[14] and Otimenyin *et al*[12]. This procedure was repeated three times to ensure complete filtration. The yellow extract obtained was then concentrated under reduced pressure at 40°C-50°C using a rotary evaporator. The extract was separated into four clear bands of different colors in the column. Elution of the extract was done with solvent systems of gradually increasing polarity using ethyl acetate, formic acid and water at the combination ratio of 5:4:1 as the mobile phase. The eluted fractions were collected in separate beakers and then poured into wash glasses and evaporated to dryness.

Drugs

Cisplatin vial (Pharmacia India) was used to induce nephrotoxicity; Cystone (Himalaya Cystone) was used as standard drug and both were procured from medical shop.

Nephroprotective activity

Ethanolic extract of powder of *curcuma longa* was studied for its Nephroprotective effect against Cisplatin induced nephrotoxicity in adult Wistar rats (200-250gm)

Phytochemical analysis

Preliminary phytochemical screening of ethanolic extract was performed to identify various phytoconstituents[9]. Results of phytochemical screening are given in Table No.1.

Animals

Swiss Albino mice (20-25gm) and Wistar rats (200 to 250gm) of either sex and of approximately the same age and weight. They were housed in polypropylene cages and fed with standard commercial pelletand wate. Before conducting each test, the animals were fasted for at least 12 h. The experimental protocols were approved by the Institutional Animal Ethical Committee.

Acute oral toxicity study

Male Swiss albino mice (20-25gm) were orally administered ethanolic extract of test drug and were observed for any symptoms of toxicity for 48h as per CPCSEA guidelines. LD_{50} was estimated by Karber's method and was found to be 2260mg/kg. Based on these results, the dosage for further pharmacological study was fixed at 250mg/kg, p.o[10].

Nephroprotective activity

Ethanolic extract of dried fruits of *curcuma longa* Linn was studied for its Nephroprotective effect against Cisplatin induced nephrotoxicity in adult Wistar rats (200-250gm)[32].

Five groups (n=6) were used to study the effect of extract against Cisplatin induced renal toxicity in rats.

Group 1 was administered with equivalent volumes of vehicle (distilled water) for 10 days, which served as normal control. Group 2, 3, 4 and 5 were administered cisplatin 5mg/ kg of body weight single dose, *i.p.* (intraperitoneal).

The blood was withdrawn on 5th day from group second and on 15th day from group third to check the persistence of renal injury.

Fourth group served as curative regimen, which was treated with 250mg/kg of ethanolic extract of *curcuma longa* and animals of group 5th were treated with cystone (standard drug) 500mg/kg along with cisplatin 5mg/kg for 5 days.

After 2 weeks of treatment, animals were anaesthetized by chloroformand sacrificed.

Blood was then collected by cardiac puncture and kidneys were dissected out immediately and transferred into 10% formalin for its histopath-ological studies[31,11].

Parameters assessed for renal function:

Body weight

The weight (in grams) of the animals was noted on the first and last day of treatment and the percentage change in body weightwas calculated.

Blood urea

Urea concentration in blood was estimated by enzymatic method using Urease enzyme kit.

Serum creatinine

Creatinine level in serum was estimated by alkaline picrate method using creatininekit.

Histopathological studies

Formalin preserved samples of kidneys from various groups were studied for histopathological changes during experiment. Sections of kidneys, stained with hematoxylin and eosin, were observed under standard microtechnique[33].

Statistical analysis

Data was statistically analyzed by Student -t test and all values expressed as Mean

± SEM. Data was also analyzed by one way Figure No.1 to Figure No.5.

RESULTS AND DISCUSSION

Histopathological study showed that cisplatin induces renal injury, as evidenced by decreased renal function in experimental animals. Table No.2 reveals that administration of cisplatin at 5mg/kg body weight produced a significant increase in blood urea, serum creatinine and protein level followed by significant decrease in body weight of the experimental animals on 5^{th} and 15^{th} day. The alcoholic extract of *curcuma longa at* the dose level of 250mg/kg and Cystone 500mg/kg was found to normalize the body weight, raised blood urea and serum creatinine.

Kidney sections of normal rats showed normal architecture of tubules, while in cisplatin treated rats (5^{th} day) showed presence of cyst and mild degenerative changes in some tubules. The kidney sections of rat treated with the cisplatin, on 15^{th} day showed degenerative chang-es in tubules with mild infiltration of leukocytes. The kidney of rat treated with ethanolic extract after 6^{th} day of cisplatin treatment showed presence of cyst in some tubules and reduction in degenerative changes. Standard drug (cystone) treated rats kidney sections showed architecture similar to normal tubules. The results of histopathology of the rats are given in Figure No.1 to Figure No.5.

Nephrotoxicity is one of the major side effects of cisplatin. Several studies have shown that cisplatin induces renal damage by free radical generation. Hence antioxidants and free radical scavengers of natural and synthetic origin might provide nephroprotection in cisplatin induced renal injury[17]. The ethanolic extract of dried fruits of *curuma longa* possesses good nephroprotective property comparable to cystone. Such an effect of extract may be due to its antioxidant activity due to the presence of flavanoids.

Further studies are required to evaluate the antioxidant and nephroprotective nature of *curcuma longa* extract in chronic renal failure models. The isolation of active principle, its evaluation in experimental model and study of mechanisms of actions will certainly be fruitful to develop new drugs. The work in this direction is in progress.

Kidney structure and functions based on different parts

S.No	Parts of the kidney	Functions
1	Hilus	Renal vein and ureter exit and the renal artery enters the kidney
2	Renal capsule	Maintain the kidney's shape and protect the kidney from damage
3	Renal cortex	Bowman's capsules, glomeruli, proximal and distal convoluted tubules and blood vessels are found.
4	Renal medulla	Loop of Henle and collecting ducts are found
5	Renal pyramids	Transport urine from the cortical or outer part of the kidney where urine is produced to the calyces in which urine collects.
6	Renal pelvis	Receives urine drained from the nephrons through the collecting ductsand papillary ducts
7	Renal artery	Blood vessel that carry oxygen-rich blood to the kidney
8	Renal vein	Blood vessel that collects deoxygenated blood from the kidney
9	Interlobular artery	Blood vessel that delivers oxygen-rich blood to the glomerular capillaries
10	Interlobular vein	Blood vessel that receives deoxygenated blood from the glomeruli and the loops of Henle
11	Nephrons	Functional units where the kidney's main functions are performed
12	Collecting duct	Collects urine and drains finally into the ureter and urinary bladder
13	Ureter	Structure which conveys urine from the kidney to the urinary bladder.

Major functions of the kidney

S.No	Basic functions of kidney	
1	Excretory functions	Excretion of nitrogenous waste products of protein metabolism Excretion of most drugs or toxins Regulation of extracellular fluid volume and blood pressure by alteringsodium excretion. Regulation of osmolality by altering water excretion Regulation of plasma electrolyte concentration within normal range Regulation of plasma pH by eliminating excess hydrogen andbicarbonate ions
2	Non-excretory functions	**Synthesis and activation of hormones** Erythropoietin which stimulate red blood cell synthesis by bone marrowRenin which control blood pressure, salt and water balance. Prostaglandins which act as vasodilators and prevent renal ischemia. *1,25*-dihydroxyvitamin D3, potent form of vitamin D which maintainbone health
		Degradation of polypeptide hormones Antidiuretic hormone, gastrin, glucagon, growth hormone, insulin,parathormone, prolactin, vasoactive intestinal poly peptide

Major Risk factors of drug induced nephrotoxicity

S.No	Major risk factors of drug induced nephrotoxicity[16]	
1	Patient-related factors	Sex- female Age - children and age over 65 yearsCo-morbidities and polypharmacy Volume depletion Reduced glomerular filtration rate Increased proximal tubular toxin reabsorption Decreased distal tubular urine flow rates Metabolic perturbations Alkaline or acid urine pHImmune response genes Gene mutations in hepatic and renal P450 system Gene mutations in renal transporters and transport proteinsAcute/Chronic kidney disease Nephrotic syndrome Cirrhosis/obstructive jaundice
2	Kidney-related factors	High perfusion rate Enhanced toxin level Biotransformation of substances to reactive oxygen speciesElevated metabolic rate of tubular cells Proximal tubular absorption of toxins
3	Drug-related factors	Prolonged dosing periods Potent direct nephrotoxic effects of the drug or compound Combinations of toxins/drugs promoting enhanced nephrotoxicityCompetition between endogenous and exogenous toxins for transporters Increasing toxin accumulation within the tubular cell Intratubular crystal precipitation of insoluble parent compound ormetabolite.

Risk factors of drug induced urolithiasis

S.No	Major risk factors of drug-induced urolithiasis	
1	Patient-related factors	Sex- Male Age-age over 40 years Co-morbidities and polypharmacy Family history Volume depletion High concentration of minerals and chemicals within the urineLiving in warm climate Drinking insufficient amount of water or excess sweating High dietary intake of animal protein, sodium, refined sugars, fructose High body mass index (BMI), large waist size and weight gainAlkaline or acid urine pH Increased calculi-induced toxin reabsorption Hyperparathyroidism, hypercalcemia and hyperuricosuria Insulin resistant states, surgical menopause, history of gout, urinary tract infections, cystic fibrosis, hypertension, gastricbypass surgery, inflammatory bowel disease or chronic diarrhoea, Crown's disease
2	Kidney-related factors	High perfusion rate Enhanced drug level which induce calculi Biotransformation of substances to reactive oxygen speciesDecreased urine volume Increased excretion of stone-forming componentsChanges in the acid-base balance (pH) Decrease in urinary citrate levels Excess vitamin D, deficiency of vitamins A or C Intratubular crystal precipitation of insoluble dug or metabolite
3	Drugs-related factors	Prolonged dosing periods Combinations of toxins/drugs promoting kidney stone formation Increase toxin accumulation within the tubular cell Urinary supersaturation of drug or its metabolite Drug -Induced metabolic calculi

Table No.1: Results of phytochemical screening

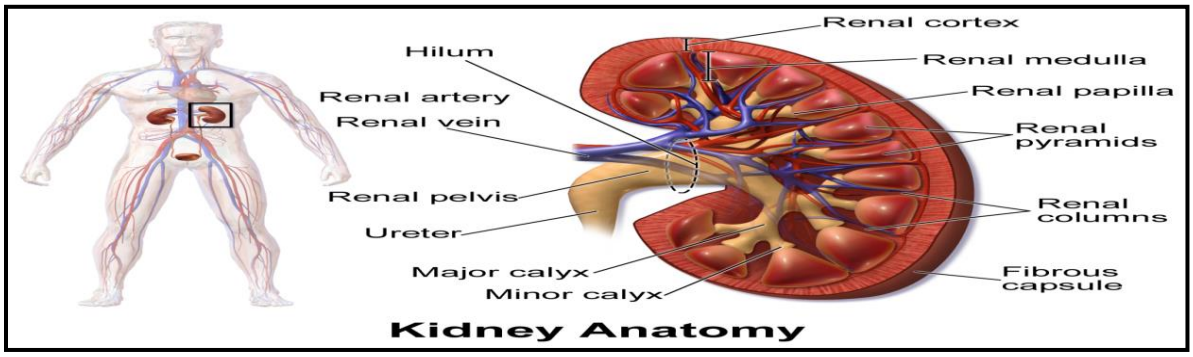
S.No	Tests	Observation	Inference
1	**Tests for Alkaloids**		Alkaloids present
	Mayer's reagent	+	
	Dragondroff's reagent	+	
	Hager's reagent	+	
	Wagner's reagent	+	
2	**Tests for sterols**		Sterols absent
	Liebermann's sterol test	-	
	Salkowski's test	-	
3	**Tests for carbohydrate and Glycosides**		Carbohydrate and glycosides present
	Molish reagent	-	
	Fehlings reagent	+	
	Barfoed's reagent	+	
	Anthrone test	+	
4	**Tests for Flavone and flavonoids**		Flavones and Flavonoids present
	Aqeuos NaOH	+	
	Conc. H_2SO_4	+	
	Shinoda's test	+	
5	**Tests for Triterpenoids and saponins**		Triterpenoids and saponins may be present
	Tin + Thionyl chloride	-	
	Foam test	+	
	Haemolysis test	+	
6	Test for tannins		Tannis absent

Grouping of animals in cisplatin-induced nephrotoxicity model

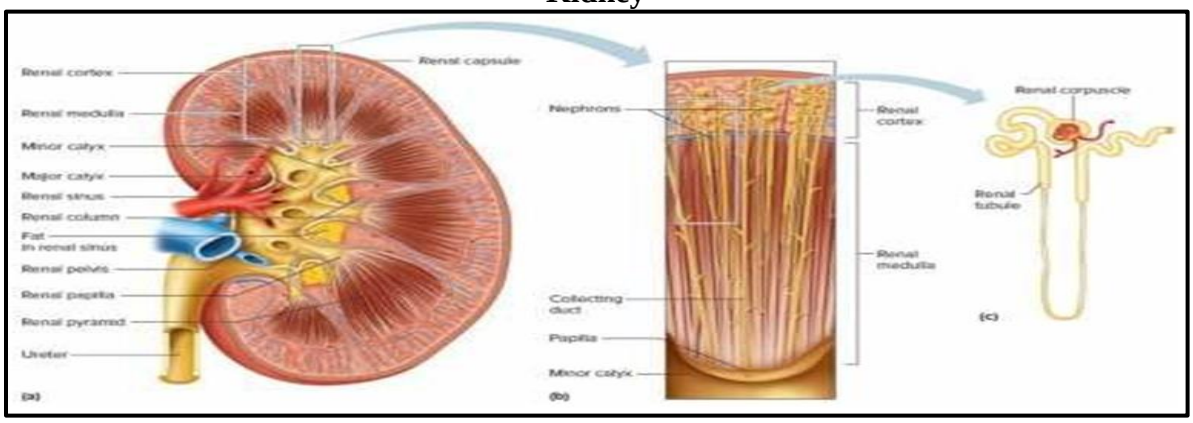
S.No	Cisplatin -induced nephrotoxicity	
1	**Group**	**Treatment**
2	Group I	Normal saline 1mL/day; for 9 days; *i.p*
3	Group II	CDDP single dose of 8mg/kg on fifth day; *i.p.*
4	Group III	CDDP+Quercetin 50mg/kg; for 9 days; *p.o.*
5	Group IV	CDDP+Extract 250mg/kg; for 9 days; *p.o.*
6	Group V	CDDP+Extract 500mg/kg; for 9 days; *p.o.*

Kidney sections of normal rats showed normal architecture of tubules

Groups	Treatment	Physical parameter	Biochemical parameters	
		Body weigh t (% change)	**Blood urea (mg/dl)**	**Serum creatinine (mg/dl)**
1	Normal control	3.45±0.22	37. 76±3 .089	1.056 ±0.049
2	Cispla tin 5th day (toxic control)	-14.47±0.165*	95.55±0.443*	1.85±0.031*
3	Cisplatin 9th day (toxic control)	- 31.23±0.371*	75.09±0.268*	1.65±0.012*
4	Ethanolic extract + Cisplatin	7.13 ± 0.102*	50.12±0 .121*	1.18±0.023*
5	Ystoe + cisplatin (Standard)	5.53 ± 0.213*	37.23±0.2 34 *	1 .089±0.021*

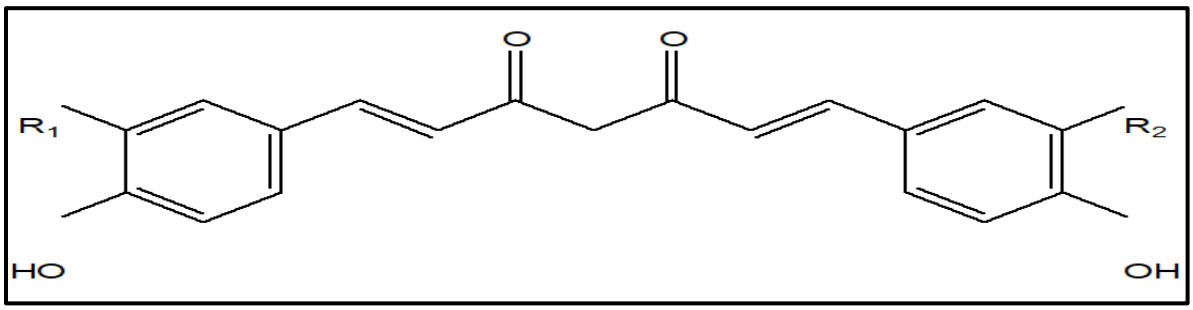


Kidney

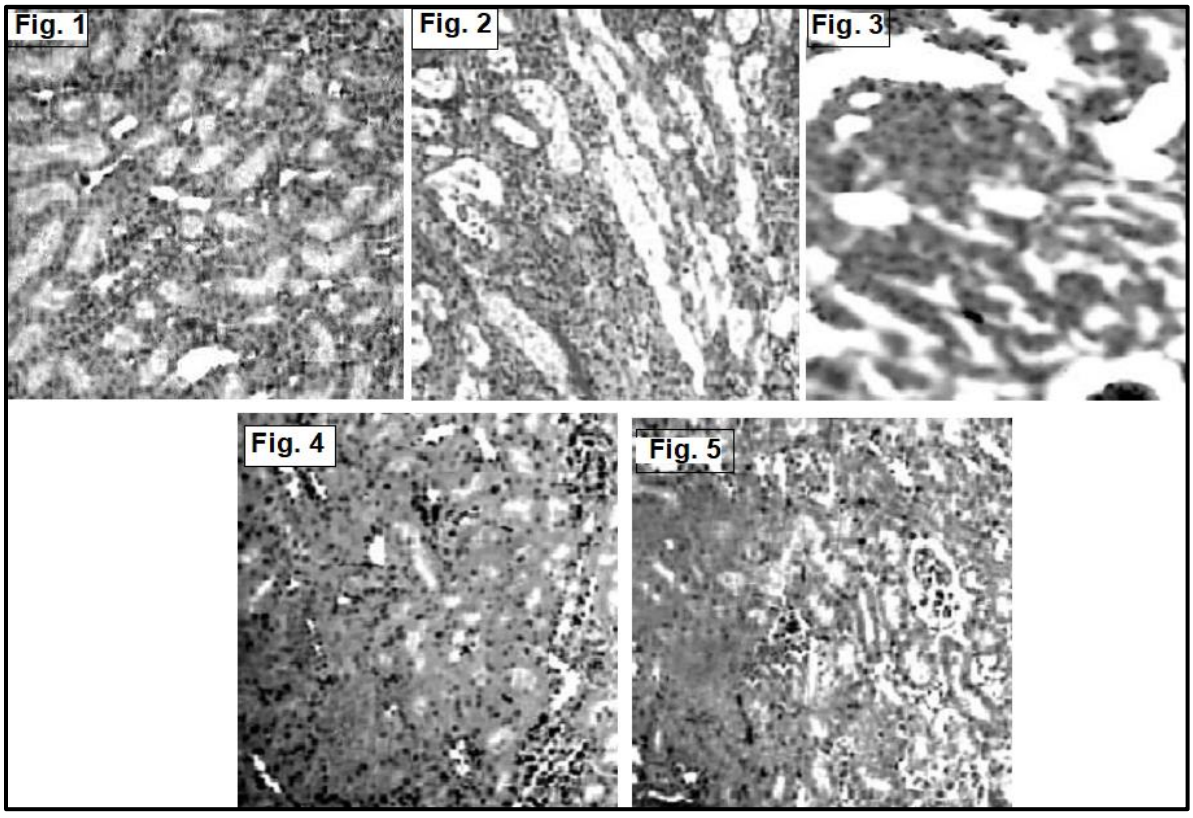


Structure of the kidney

Turmeric



Curcumnia	**OCH_3**	**OCH_3**
Desmetoxicurcumnia	**H**	**OCH_3**
Bisdesmetoxicurcumnia	**H**	**H**



Data was also analyzed by one way Statistical analysis

CONCLUSION

The ethanol extract of *Curcuma longa* was able to protect kidney damage due to toxic dose of cisplatin. Turmeric is an aromatic spice, traditionally used as a food additive in curries, giving them their distinctive flavor and color. It has been used in traditional medicine for the treatment of various diseases as discussed above. The present investigation was an attempt to explore few of the diverse pharmacological properties of turmeric. The main activities like anti-inflammatory, antioxidant, antimicrobial, anticancer and anti-carcinogenic, antivenom, anti-HIV, wound healing, photo-protector has been described. The fast growing research on turmeric and its metabolites clearly confirms the versatility and flexibility of curcumin for structural modifications. However, the actual role of different functionalities in curcumin in influencing its special physical chemical properties and pleiotropic effects of natural and synthetic curcuminoids is far from understood. The extensive survey of the literature revealed that Curcuma longa is highly regarded as a universal panacea in the herbal medicine with diverse pharmacological activity spectrum. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible of the various activities of the plant. Hence, extensive investigation is needed to exploit their therapeutic utility to combat diseases. This review describes various approaches that have been undertaken to prove the biological importance of turmeric. The result was supported by histopathological study which showed no damage on kidney organs. However, further studies are required to elaborate molecular mechanism of Curcuma longa as nephroprotective agent.

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CONFLICTS IF INTEREST

The author declares no conflict of interest.

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