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Review on Antioxidant, Anticancer and Hepatoprotective Activity of *Curculigo orchioides*

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ABSTRACT

The main aim of the present review is to investigate the role of antioxidant, anticancer & hepatoprotective activity of *Curculigo orchioides*. *Curculigo orchioides gaertn* is one of the highly useful plant in indigenous system of medicine belongs to Amaryillaceae family. Antioxidants are chemicals (both naturally occuring and manmade) that can prevent or slow cell damage. Any compound that can donates electrons and counteract free radical has antioxidant properties. Natural antioxidants are many founds in friuts, vegetables, marine plants and some vitamins. Cancer remains as one of the major cases if death throughout the world. In cancer cell grow and divide in an uncontrollable manner. Cancer may spread to other parts of the body. There are many factors which leads to cancer. Many herbs, have been used for treatment purpose stage have shown the presents of chemo preventive agent in daily consumed plan based diet. They are phytochemicals, phenolics(tannins, liganans, flavanoids), caratenoides, glucosinolate. These agent are found in fruits, vegetables, herbal extract and beverage such as tea, coffee. These phytochemicals fullfil the requirement of chemopreventive agent, such as toxicity, efficacy against most type of cancer. The liver plays a major role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, those used in laboratories and industries, natural chemicals and herbal remedies. Can also caused hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. There are several herbs/herbal formulations claimed to possess beneficial activity in treating hepatic disorders. Number of plant drugs are included in several ayurvedic formulations which are proven as antioxidant, anticancer and hepatoprotective activity. The review reveals that the extract of *Curculigo orchiodes* shows potent scavenging activity. The extract of *Curculigo orchiodes* significantly reduced the elevated level of serum enzymes like SGOT, GPT, SALP and total bilirubin level. These marker enzymes in ccl4 treated rats. this implies that the extract tends to prevent liver damage, preserves the integrity of the plasma membranes and hence rest.

INTRODUCTION

Curculigo orchioides gaertn (Black musli or Goldeneye grass) is one of the highly useful plant in indigenous system of medicine, belongs to Amaryillaceae family. It was first introduce in "Chark samhita of agnivesha", the epic treatise of the medicine school of thought of the Hindu system of medicine and narrated as an ingredient of a cigar to alleviate cough.

Curculigo orchioides Gaertn is a small herb, up to 30cm high with tuberous root stock, occurring wildly in subtropical Himalayas and almost all parts of India. Drug is collected from two year old plant. The active compounds that have been reported are flavones, glycoside, steroids, triterpenoids and other secondary metabolites. β-sistosterol and crystalline needles of sapogenin has also been detected.

The rhizomes of this plant are sweet, cooling, diuretic, aphrodisiac, viriligenic and tonic which can be used against hemorrhoids, leucorrhoea, pruritis, skin disease, asthma, bronchitis and jaundice. It is also used as antioxidant, spermatogenic, hepatoprotective, immunostimulant, anticancer, antibacterial, antiosteoporotic and hypoglycemic.

CHEMICAL CONSTITUENTS

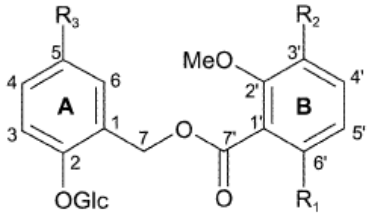
Some of the chemical constituents found in *Curculigo orchioides* include:

- Mucilage
- Phenolic glycosides
- Saponins
- Aliphatic compound cycloartane glycosides.

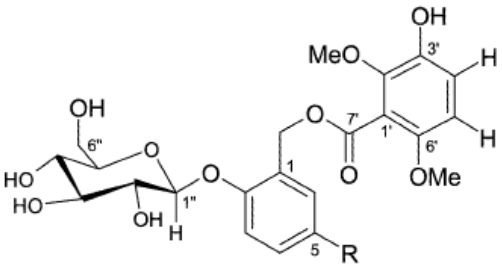
In the rhizomes of *Curculigo orchioides* were found phenolic glycosides. Others as:

- Resin
- Tannin
- Oxalate of calcium
- Glucosides curculigoside A
- Curculigoside B

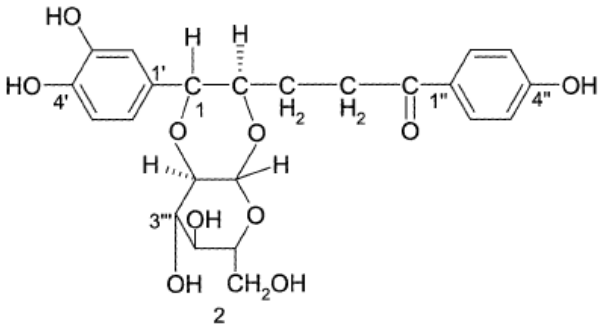
- Curculigoside C
- Curculigoside D
- Orcinol glucoside
- Orcinol-1-O-beta-D-glucopyranosyl-(1->6)-beta-D-glucopyranosides
- Curculigoside
- Odoroside A
- Odoroside B
- Odoroside C
- 2,6-dimethoxyl benzoic acid
- Syringic acid
- Curculigo saponin



	R_1	R_2	R_3	
1	OMe	H	OH	Curculigoside A
2	OH	H	OH	Curculigoside B
3	OMe	OH	OH	Curculigoside C
4	OMe	OH	H	Curculigoside D

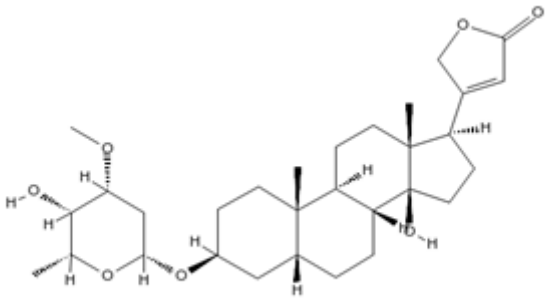


1. R = H
3. R = OH



2

Orchioside A (1)
Orchioside B (2)
Curculigoside C (3)



Odoroside A

Distribution

Curculigo orchioides grows in the shady forests area of Asia. This small herb is widely distributed and shows prostate growth in moist and humus rich soil. The optimum spacing between two tubers are 10X10cm. It is widely found in all parts of India from near sea level to 2300m altitude. It has been cultivated and distributed in the subtropical Himalayas regions, Kumaon eastwards, Bengal, Assam, Konkan, Khasi hills, Kanara, the western peninsula and Tamil Nadu extending south as far as Cape Comerin. It is also grow in Japan, Malasia, Sri Lanka and Australia. The demand of the raw materials and derivatives of the *Curculigo* for the herbal drugs industries is satisfied mainly from the wild source. In the CAMP workshop at IIFM.

Curculigo orchioides was listed in the IUCN category of "LOWER RISK, near threatened"[1].

MORPHOLOGY

It is a perennial herb about 30cm in height with a short or elongated rootstock bearing many fleshy and lateral root which are blackish brown internally and cream externally.

- **Rhizome**

Drug occurs in transversely cut pieces of 2.5 to 5cm in diameter; external surface is blackish brown, with wrinkles, rootlets, root scars, nodes, internodes and transverse cracks, cylindrical in shape, straight to staightly curved, internal surface is cream colored; taste is mucilaginous and slightly bitter.

- **Leaf**

Leaves are simple 15-45cm long crowded on the short stem, sessile or short petiolate with sheathing leaf base and often produce adventitious buds at the tip when in contact with soil.

- **Flower**

Flowers are bright yellow in colour and the upper few are male flower which are smaller in size, while the lower once are bigger and may be female. Inflorescences umbel-like racemes, 4-6-flowered. Anther 2-3mm; ovary narrowly oblong in shape and of 7 mm, pilose; stigma lobes longer than style.

- **Fruit**

Fruit is capsule, oblong glabrescent with a slender beak and spongy septa, 1.5-2cm long and 8mm broad. It contain 8 seeds which are globose, size 1-2mm, black, deeply grooved in wavy lines [2-3].

Powder microscopic characters

Powder microscopic character of rhizome study shows that it is light brown, slightly bitter in taste contain annular and spiral xylem vessels, simple, round to oval, starch grains measuring 4 to 21 µ in diameter. Compound starch grains having 2 to 4 components and a few acicular crystals of calcium oxalate[4].

Pharmacological activities of *Curculigo orchioides:*

The pharmacological activities of *Curculigo orchioides* are oxytocic activity[4], hepatoprotective activity[6], antioxidant activity[7], hearing Loss[8], immunomodulatory activity[9], aphrodisiac activity[10], spermatogenic activity[11], antidiabetic activity[12-13], estrogenic activity[14], antiosteoporotic activity[15-16], antiasthmatic activity[17], antibacterial activity[18], analgesic activity[19], anticonvulsant activity, antihistaminic activity, antitumor activity, inhibitory activity, wound healing activity, anti-inflammatory activity.

INTRODUCTION

Raghunathan *et al.*, reported that various vaidyas and traditional tribal's prescribed *Curculigo orchioides* in various systems of medicine, especially in Indian systems, for long periods. Herb-properties in various doses and combinations for the treatment of a number of

diseases such as antidiabetic, aphrodisiac, asthma, bronchitis, demulcent, diarrhea, diuretic, dyspnoea, dysuria, gonorrhea, hydrophobia, indigestion, jaundice, leucorrhoea, menorrhagia, menstrual derangements, ophthalmia, pains in the joints, piles, tonic and vomiting have been described[20].

Curculigo orchioides Gaertn contain three steroids, sitosterol, stigmasterol and yuccagenin. Lycorine is the only alkaloid isolated and known so far in Curculigo orchioides Gaertn. Five phenolic compounds have been isolated and characterized from Curculigo orchioides Gaertn. These are curculigoside (5- hydroxy-2- O-D-glucopyranosyl benzyl-2, 6-dimethoxy benzoate), curculigine A, orcinol glucoside, corchioside A19 and flavanone glycoside–I (glycoside–5, 7- dimethoxy-dihydromyricetin3-O-L-xylopyranosyl-(4-1)-Dglycopyranoside). A number of fatty acids have been isolated from root oil of plant by gas liquid chromatography. They are palmitic, oleic, linoleic, arachidic and behenic acid.

Rajagopalan *et al.*, (1994) *Curculigo orchioides Gaertn* of Amaryllidaceae family is a herbaceous tuberous geophilous perennial with rootstock bearing several fleshy lateral roots (rhizomes). It is widely distributed in India. The rhizomes of this plant possess medicinal properties and are sweet, cooling, diuretic, aphrodisiac, viriligenic and tonic which can be used against hemorrhoids, leucorrhoea, pruritis, skin diseases, asthma, bronchitis and jaundice etc[21].

ANTIOXIDANT ACTIVITY

Halliwell B *et al.*, (1990) The role of medicinal plants in promoting health, reducing risks of illness and for treating ailments has garnered increasing interest amongst researchers across the world in seeking evidence to justify the health benefits of these plants. Many diseases are now known to be caused by oxidative stresses and emerging bacterial activities. Consumption of antioxidants is associated with reduced risks of many oxidative diseases such as cardiovascular disorders, diabetes mellitus, cancer and rheumatic arthritis[22].

According **Pradnya Onkar *et al.***, the antioxidant activity of hydroalcoholic extract of *curculigo orchioides* gaertn was studied. Antioxidant activity are performed by DPPH method and hydrogen peroxide scavenging activity. DPPH Assay (1,1 diphenyl 2, picryl hydrazyl): 0.3ml solution of DPPH in 100% ethanol was prepared 5ml of this solution +1ml of the fraction dissolved in ethanol at different concentrations (50-250 µg/ml), mixture was shaken and allowed to stand at room temperature for 30 min. Absorbance was measured at

517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and compared with that of Butylated hydruxy toluene, which was used as the standard. Determination of reducing power: 2.5ml of solution of different concentrations of extract (50, 100, 150, 200, 250 µg/ml). Absorbance was measured at 700nm. Hydrogen peroxide scavenging activity: Hydrogen peroxide 2mm/L solution prepared with standard (PO4 buffer Ph-7.4). Different concentrations of extract (50, 100, 150, 200, 250µg/ml) prepared in distilled water.1ml of solution of different concentrations of extract (50, 100, 150, 200, 250 µg/ml). Absorbance was measured at 230nm against blank solution containing PO4 Buffer without hydrogen peroxide. The results revealed that the extract shoes potent scavenging activity when compared with standard Butylated hydroxyl toluene. These active constituents may be responsible for the observed antioxidant activity. Further study on the active components may provide a better understanding about plant with a goal of elucidating their active potential compound[23].

From the work of **K V Ratnam *et al.,*** the antioxidant activity of ethanolic root extract of *Curculigo orchioides* was determined by three methods. DPPH (2, 2-diphenyl 1-picryl hydrazyl) Free Radical Scavenging Activity: The free radical scavenging activity was followed by DPPH method. 0.1M solution of DPPH in methanol was prepared. Gallic acid was taken as reference standard. Different concentrations of extract (50.0, 100.0, 300.0, 500.0 µ g/ml) and standard (1.0, 2.5, 5 µg /ml) were prepared using methanol. 1.0M DPPH and methanol were used as blank. The absorbances were measured at 517nm. Finally, the % inhibition was calculated.

Where A0 is the absorbance of the blank (containing all reagent except the sample extract), and A1 is the absorbance of sample extract. The anti-oxidant activity of ethanolic root extract of *Curcurligo orchioides* was expressed as IC_{50}. The IC_{50} value is defined as concentration in (µg/ml) of extract that scavenges DPPH radical by 50%. Reducing Power Assay: Different concentrations of extract (50.0, 100.0, 300.0, 500.0 µg/ml) and standard Gallic acid (1.0, 2.5, 5.0 µg/ml) were prepared using distilled water. Absorbances were measured at 700nm.

Phospho Molybdenum Assay: Different concentrations of extract (50.0,100.0, 300.0,500.0 µg/ml) and standard Gallic acid (1.0, 2.5, 5.0 µg/ml) were prepared using distilled water. The absorbance of the solution was measured at 695nm using spectrophotometer. The result obtained indicates the significant antioxidant activity in all three methods and the results were compared with standard reference drug Gallic acid[24].

In the study of **M. R. Venukumar *et al.*,** of the antioxidant activity of methanol extract of rhizomes of *Curculigo orchioides* (MEC) was investigated using carbon tetrachloride (CCl_4)-intoxicated rat liver as the experimental model. In the present study, elevated level of thiobarbituric acid reactive substances (TBARS) and diene conjugates (CD) observed in CCl_4- treated rats indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. TBARS produced as byproduct of LPO that occurs in hydrophobic core of bio-membranes. The significant decline in the concentration of these constituents in the liver tissue of CCl_4 + MEC administered rats indicates anti-lipid peroxidative effect of *Curculigo orchioides*[25].

ANTICANCER ACTIVITY

Swamy MV *et al.*,(2002) traditional medicinal plants and practices have remained as a component of health care system of many societies in spite of the availability of well established alternatives. Apoptosis plays a central role in tumor development and it has been hypothesized that lack/failure of apoptosis leads to the development of tumors, including colon tumors. Thus induction of apoptosis in tumor cells is an effective approach to the regulation of tumor growth[26].

Fruehauf JP *et al.*, (2007) Recently chemotherapeutic agents are used with the combination of phytochemical agents. This would enhance the efficacy while reducing toxicity to normal tissues. Free radicals react with purines, pyrimidine, and chromatin protein leading to base modifications, unstable genomes and genetic alterations. These transformed cells have altered levels of cell cycle and apoptosis signalling molecules thereby resulting in uncontrolled cell proliferation and tumour formation[27].

According to **Raaman N *et al.***, study, *in vitro* antitumor activity of methanoic extract of *Curculigo orchioides* using Hep2 cell lines respectively at various doses ranging from 10 to 800 µ g in the confluent culture. The results shows that the antimicrobial and antitumor activity of different fractioned extracts of Klaimusli is due to presence of saponins, which are the glycosides present in plant. These plant glycosides are the polar compounds, therefore the methanolic extracts is showing the maximum activity. The cell proliferation activity was qualified on MCF-7 cell line, by using positive and negative controls (positive control-17-beta-estradiol; negative control-culture medium only).

HEPATOPROTECTIVE ACTIVITY

M. R. Venukumar *et al.*, (2002) Hepatic damage as evidenced by a rise in the levels of AST, ALT, ALP and GGT in serum, and also changes observed in other biochemical parameters in serum and liver showed a tendency to attain near normalcy in animals co-administered with MEC. The normal values for AST (IU/L), ALP (IU/L), protein (g/100 ml) and total lipids (mg/100 ml) in serum (i.e., 21.24, 71.04, 6.72 and 136.54 respectively) were found to alter towards values 33.61, 128.11, 4.83 and 266.91 in hepatotoxic rats. These parameters attained near-normal values (i.e., 23.82, 80.3, 6.22 and 152.24 for AST, ALP, protein and total lipids respectively) in MEC co-administered rats. Profound steatosis, ballooning degeneration and nodule formation observed in the hepatic architecture of CCl_4 treated rats were found to acquire near-normalcy in drug co-administered rats, thus corroborating the biochemical observations[28].

Rao *et al.*, (1996) suggested the anti-inflammatory and hepatoprotective activities of *Curculigo orchioides*. They observed hepatoprotective activity against rifampicin-induced hepatotoxicities and also isolated curculignin A and *Curculigol* and screened for their anti-hepatotoxic activity against thioacetamide and galactosomine-induced hepatoxic[29].

Babu G *et al.*, (2013) it conducted to evaluate the hepatoprotective activity of ethyl acetate extract of rhizomes of *Curculigo orchioides*. The extract at the doses of 200 mg/kg and 400 mg/kg b.wt. was tested for its hepatoprotection, by inducing hepatotoxicity with CCl4 in wistar albino rats and using silymarin (100 mg/kg) as the reference standard. Biochemical parameters like SGOT, SGPT, SALP and serum bilirubin were determined to assess the hepatoprotective effect. The extract has shown significant hepatoprotection in albino rats in reducing SGOT, SGPT, SALP and serum bilirubin levels.

Praveen Kumar *et al.*,(2019) Ethanolic and aqueous extracts prepared from rhizomes of *Curculigo orchioides* as herbal medicines were evaluated against acetaminophen-induced hepatotoxicity in cockerels. Acetaminophen at 500 mg/ body weight orally was given to induce hepatocellular damage. Cockerels given with ethanolic extract of *Curculigo orchioides* at 70 mg/kg body wt and acetaminophen revealed restoration of Hb, PCV, TEC, TLC and lymphocytes and heterophils as well as total protein, albumin and globulin, glucose, cholesterol, bilirubin and activity of AST, ALT, ALP and LDH. The biochemical results were parallel to the histopathological analysis of liver sections as treated birds clearly showed normal hepatic cells and central vein thereby confirming hepatoprotective activity. These

findings had suggested that *Curculigo orchioides* is a promising product in protecting the liver against toxic injury via the restoration of haematalogical and biochemical parameter. Aqueous extract showed least activity. Ethanolic extract showed presence of alkaloid, flavonoid, glycosides, Protein, reducing sugars, resin, saponins and sterol[30].

From the work of **D Bargavi *et al.***, to investigate the possible hepatoprotective activity of ethanolic extract of *curculigo orchioides (EECo)* against paracetamol ethanol included hepatotoxicity in in-vivo studies. Methods are Acute Toxicity studies. The extract was administered orally at doses from 200-2000mg/kg. There were no signs of toxicity and mortality was observed up to 2000mg/kg[31].

Paracetamol induced hepatotoxicity, ethanol induced hepatotoxicity. In both the methods animals were sacrificed 24hr after the last treatment. Biochemical investigations were carried out. Liver was dissected out and used for histopathological studies. This test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals. Rats treated with ethanol developed a significant hepatic damage observed as elevated serum levels of hepato specific enzymes like SGPT, SGOT and Albumin, Total protein and Creatinine when compared to normal control. Pre-treatment with silymarin, EECO had showed good protection against ethylene glycol induced toxicity to liver. Result indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals.

From the data of **M S Venukumar *et al.***, hepatoprotective effect of the methanolic extract of *Curculigo orchioides* In CCl4 – treated male rats by Experimental induction of hepatic damage. Evaluation was done by Biochemial estimations and Histopathology. A few photomicrographs of representative types were also taken. The result indicates that food consumption and weight gain significantly increased in group 3 animals as compared to other groups. In group 2 rats there was a lesser weight gain as compared to group 1 animals. All the marker enzymes, viz., AST, ALT, ALP and GGT registered enhanced activity in CCl4-treated rats as compared to control group in MEC co-administered group, the levels of these enzymes were found retrieving towards normalcy. The total protein concentration of the serum and liver was lesser in group 2 animals when compared with normal control and it attained an almost normal value in group 3 rats.

The level of total lipids, triglycerides and cholesterol in serum as well as liver recorded significant increment in CCl4-administered rats as compared to those of group 1. All these

biochemical changes showed signs of returning towards normalcy in group 3 animals. There was a significant decline in the concentration of phospholipids in liver tissues of CCl_4-treated rats as compared to normal control. In group 3 animals phospholipid concentration attained normalcy[32].

DISCUSSION

In this study discussed, mainly activities of *Curculigo orchioides*. The different parts of Kalimulsi (*Curculigo orchioides*) were fractionated with different solvents and screened for their antioxidant, anticancer, hepatoprotective activity.

Antioxidants are helpful in the defense mechanism of body against different pathogens. The use of plant derived antioxidants is helpful against many degenerative diseases like Parkinson's, Alzheimer and Cancer. This evaluation gives an overlook to the different methods used to determine antioxidant capacity of different antioxidants. The explanation of analytical performances and principles of different methods used for the determination of antioxidant capacity involve techniques are discussed.

In this study antioxidant was investigated using carbon tetrachloride (CCl_4)-intoxicated rat liver as the experimental model. The hepatotoxic rats were administered MEC for 90 days (daily, orally at the dose of 70 mg per kg body weight). Lipid peroxidation (LPO) in CCl4 - intoxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS) and diene conjugates (CD), and also a distinct diminution in glutathione (GSH) content in the liver. In CCl_4 + MEC – treated rats these activity of methanol extract of rhizomes of *Curculigo orchioides* (MEC) biochemical parameters attained an almost normal level. The decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GRD) in CCl_4 –intoxicated rats, and its retrieval towards near normalcy in CCl4 + MEC- administered rats revealed the efficacy of MEC in combating oxidative stress due to hepatic damage. Elevated level of glutathione transferase (GTS) observed in hepatotoxic rats too showed signs of returning towards normalcy in MEC co-administered animals, thus corroborating the antioxidant efficacy of MEC. The findings provide a rationale for further studies on isolation of active principles and its pharmacological evaluation.

Antitumor activity was screened against a human breast cancer cell line (MCF-7). Methanolic extract showed maximum activity due to the saponins present.

The antioxidant and anticancer activity of ethyl acetate extract and nanoparticles synthesized from *Curculigo orchioides*. The ethyl acetate extract and nanoparticle synthesized from *C. orchioides* were used in various antioxidant assays such as DPPH, hydroxyl radicals, hydrogen peroxide and nitric oxide radical scavenging activities. The synthesis of silver nanoparticles was characterized by UV-Vis spectrophotometer, X-ray diffractometer (XRD) and Scanning Electron Microscope (SEM). The maximum growth inhibitory effects (66.12% and 71.28%) on MCRF-7 cell line were observed in ethyl acetate extract and nanoparticle at 80µg/ml concentration after 48 hr treatment.

The effect of *Curculigo orchioides* rizhomes extract+ethanol (combined) on adult male rat liver metabolism was assessed in the present study and compared with control and ethanol alone treated rats. The administration of ethanol accelerates the glycogenolysis and drastically reduced the hepatic glycogen content. The hepatic transaminase activity was swayed by ethanol treatment and reverted to normalcy by combined treatment. Elevated levels of serum enzyme are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. The reversal of altered transaminase activities to normal by plant extract supplementation suggest its hepatoprotective action.

CCl4 – mediated hepatotoxicity was taken here as the experimental model for liver injury. It has been established that CCl4 is accumulated in hepatic parenchymal cells and metabolically activated by cytochrome P-450 dependent monooxygenase to form a trichloromethyl free radical (CCl3•) which alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides leading to liver damage.

It can be said that methanol extract of rhizomes of *C. orchioides* exhibit a liver protective effect against CCl4 - induced hepatotoxicity and possessed anti-lipid peroxidative and antioxidant activities. Efforts are in progress here to isolate and purify the active principle involved in the hepatoprotective efficacy of this medicinal plant.

CONCLUSION

Number of plant drug are included in several Ayurvedic formulations which are as antioxidant, anticancer and hepatoprotective activity. This implies that many of the other plant drugs are yet to be investigated for its safe use in human beings. Such a sumptuous

plant drugs treasure from Ayurveda and herbalism must be well established and protected too.

The review reveals that the extract of *Curculigo orchioides* shows potent scavenging activity. When compared with standard Butylated hydroxyl toluene and gallic acid. The plant extracts contains flavonoids, glycosides, saponins and some amount of phytosterols. These active constituents alone or in combination may be responsible for the observed antioxidant activity.

The cytotoxic activity of *Curculigo orchioides* was evaluated on human cervical cancer cell lines (HeLa). HeLa cell is a cell type in an immortal cell lines used in scientific research. It is the oldest and most commonly used human cell line.

The extract of *Curculigo orchioides* significantly reduced the elevated levels of serum enzymes like SGOT, SGPT, SALP and total bilirubin level these marker enzymes in CCl4 treated rats. This implies that the extract tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels.

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