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Review Article

Genus Rubia: Therapeutic Effects and Toxicity: A Review

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Abstract

The Rubiaceae family taxonomic classification is complex. Genus Rubia with 70 species belongs to Rubieae tribe of Rubioideae subfamily of the Rubiaceae family. These species have been widely distributed and cultivated around the world, and are mainly concentrated in the tropics. The effects of bioactive metabolites of various parts, particularly roots of Rubia species have been thoroughly examined, and their pharmacological and toxicological effects have been described. Antioxidant, anti-inflammatory, antitumor, antidiabetic, anti-arthritic, antiseizure and antimicrobial effects as well as toxicological properties of Rubia species have been previously reported. This study was conducted as a literature survey of various species of Rubiaceae published from 1992 to 2020. Moreover, their toxic and protective effects on living organisms were summarized.

Keywords: Rubiaceae, Rubieae, Rubia, Toxicity, Protective effects

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Introduction

As a family of flowering plants, Rubiaceae with subfamily Rubioideae has a genus named Rubia with more than 70 species from America, Europe, Africa and Asia as well as about 15 species reported from India. Some of the most common variants are *Rubia akane*, *Rubia tinctorum*, *Rubia cordifolia linn* and *Rubia peregrine*. This genus is also known as madder. Having alizarin component, the long, cylindric and tortuous roots of Rubia are red. Terpenes and naphtoquinones as well as anthraquinones (AQs) have been largely isolated from roots of Rubia. Other compositions are iridoid, carbohydrates and bicyclic hexapeptide (1). Rubia species have been widely used in medicine and applied in dyeing industry. It is considered as a source of food additives. For instance, it is used as a colorant and also a flavoring substance (2). Traditionally, Rubia extracts have been applied for skin care and urinary tract disorders. Although anticancer, anti-inflammatory, antioxidant, antimicrobial and spasmolytic activities of Rubia

extracts were reported (3-6), some conflicting evidence have also been reported regarding toxicities induced by it (7-10). Along with these features, Rubia cell lines have been used to study the mechanisms of synthesizing of antraquinones as well as to be transformed to increase the amounts of some ingredients such as AQ (11). In this review, pharmacological and toxicological effects of Rubia on various human body organs will be discussed.

Methodology

A thorough literature survey related to Rubia has been conducted up to May 2020. Several offline and online sources have been taken into consideration. The main sources of data collection were Sciencedirect, Embase, Medline, Scopus and PubMed using the following terms: "Rubiodeae, Rubia, chemistry, anthraquinones, antioxidant, anti-inflammatory, antitumor, antidiabetic, anti-arthritic, antiseizure, antimicrobial, and toxicological."

Genetic Manipulation in Rubiaceae Plants for Increasing the Synthesis of AQs on an Industrial Scale

Rubia species produce phytoalexins – AQs, which could be cytotoxic and also exert antioxidant and antifungal effects (5, 12). Cytotoxicity of AQs makes them a good candidate to be considered as anticancer agents (8). Hence, it could be useful to develop plant cells cultivation techniques and methods aimed to increase the synthesis of AQs in Rubia plants on an industrial scale.

Several approaches have been evaluated in terms of metabolic engineering of AQ biosynthesis. The first one utilizes the heterologous overexpression of key branch point enzymes of AQ metabolism. For example, expression of the bacterial isochorismate synthase (ICS) gene, which encodes an enzyme that catalyzes a key step in AQ biosynthesis (13), resulted in 20% increase of total AQs content in transgenic root cultures of *Rubia peregrina* (14). However, this classical method of genetic engineering was not always effective, particularly in those cases where distinct and often unknown limiting determinants prevented pathway activation (15). Thus, overexpression of the *Catharanthus roseus ICS* gene in cell cultures of this plant did not lead to increased accumulation of the isochorismate pathway metabolite derivatives (16). Besides, AQ contents in *Morinda citrifolia* cell lines overexpressing sense and antisense copies of *C. roseus ICS* gene were also similar (17).

Genetic transformation with different strains of wild-type *Agrobacterium rhizogenes* results in obtaining the so-called hairy roots cultures and often leads to unspecific activation of secondary metabolite production (18). In particular, *A.rhizogenes*-transformed *R. peregrina* roots showed two-fold increase of total AQs content, with alizarin as a major representative compound (19). Being the main pathogenic determinants of *A. rhizogenes*, *rol* genes are capable of inducing the biosynthesis of secondary compounds even when overexpressed individually under both native and strong virus promoters (20). In case of AQs, *rolB* and *rolC* genes act immediately after transformation (21), while *rolA*-mediated activation becomes more pronounced after long-term cultivation (11).

Phytoalexins production could be regulated by phosphorylation of the main enzymes in their biosynthetic pathways via different isoforms of Ca^{2+}-dependent protein kinases (CDPKs) (22). Heterologous expression of the mutant constitutively active *Arabidopsis thaliana CPK1* gene (also known as Ak1-6H) in *R. cordifolia* caused a 10-fold increase in AQ content compared with untransformed culture (23). Overexpression of native AtCPK1 also provoked AQ production in transgenic *R. cordifolia* callus. However, its stimulatory effect was approximately 4 times lower compared with mutant AtCPK1 isoform (24).

In addition to genetic tools, AQs accumulation could also be induced by the addition of chitosan in *R. tinctorum* cell line via the activation of PLC\PKC, PI3K, MAPK – signaling pathways (25). Such elicitors as salicylic acid and methyl jasmonate, but not ethephon, also strongly elevate AQ accumulation in *R. cordifolia* callus lines (26).

Antitumor Activity

Antitumor Rubiaceae-type cyclopeptides (RAs) have been isolated from the chloroform fraction of the methanol (MeOH) extract of roots of Rubiaceae. Among them, RA-VII possesses anticancer activity and has been considered to be studied in clinical trials. Anticancer activity of these cyclopeptides could be related to the impeding of protein synthesis and

induction of G2 arrest. RA-XXIII and RA-XXIV are other bicyclic hexapeptides found in the roots of *Rubia cordifolia Linn.* (27, 28).

Multiple and sequential fractionation and finally crystallization of MeOH extract of the roots of *R. cordifolia* with Ethyl acetate and CHCl3 using column chromatography give crystals of crude RAs specially RA-I, RA-XII, RA-XXIII and RAXXIV. Among them, RA-VII has been isolated from Rubiaceae plants sucha as *Rubia akane nakai* and *Rubia cordifolia linn*. Although the exact mechanisms of the anticancer activity of these cyclopeptides have not yet been completely understood, it might be due to the induction of G2 arrest and/or disruption of protein synthesis with ribosomes. Among these peptides, RA-VII has been considered as an anticancer agent for phase I clinical trials in Japan. O-seco-RAXXIV is another cyclic hexapeptide isolated from the roots of *Rubia cordifolia linn*, and it seems that it is the precursor of RA-XXIV (29). Dimeric peptide, RA-dimer B, is another cytotoxic RA that showed cytotoxicity against human colon cancer cells, human leukaemia cells and human renal cell carcinoma (30). Other RA bicyclic hexapeptides RA-XXV and RA-XXVI isolated from the roots of *Rubia cordifolia linn* are cytotoxic against human colon cancer cells and human leukemia cell lines (31). Purpurin derived from Rubia tinctorum L. was examined with regard to selective activity on cell adhesion, proliferation, and migration of tumor—melanoma—cells. Purpurin and an aqueous extract of *Rubia tinctorum L*. had tumor growth inhibitory activity in melanoma cell lines with distinct metastatic capability, and their impacts could not be altered and were independent to apoptosis (5).

Effects of Rubia on Kidney

Protective Effects

Acute renal injury might be characterized by an increase in serum uric acid, BUN, and creatinine. Anti-inflammatory and antioxidant activities of *Rubia yunnanensis* seem to be related to natural cyclopeptide RA-XII. RA-XII has protective effects against Lipopolysaccharide (an endotoxin) induced acute renal injury which is because of anti-inflammatory effects attributed to the RA-XII-induced increase in antioxidant enzymes reducing reactive oxygen species (ROS) formation such as catalase (CAT) and superoxide dismutase (SOD). Moreover, it reduces glutathione (GSH) levels and decreases malonedialdehyde (MDA) which is a marker of lipid peroxidation (LPO). Moreover, the main pathways of inhibition of inflammatory response and oxidative stress caused by Rubia are thought to be by blocking IκBα/NFκB and MAP Kinases modulated by hemeoxygenase (HO)-1 and Nrf2 pathway. Moreover, RAX II could reduce the critical biomarkers of oxidative stress and carcinogenesis 8-hydroxy-2' -deoxyguanosine (8-OHdG) (32).

Rubia cordifolia linn hydro-alcoholic root extract has been used for the treatment of bladder stones (urolithiasis) in the early stage of stone development. Hyperoxaluria plays a significant role in urinary calculi which are mainly composed of phosphate and oxalate salts of calcium. The extract reduced oxalate excretion. Another adverse effect of elevated oxalate concentration in urinary tract is increase in lipid peroxidation which was significantly prevented by the extract. Another factor affecting urolithiasis is hypocitraturia which was turned to be normal after using the extract. Another risk factor of stone formation is low urine level of magnesium. Rubia extract restores magnesium as an inhibitor of stone crystallization. Urinary phosphorus and uric acid excretion which is observed in urolithiasis were also reduced by Rubia extract. Eventually, the nephroprotective effect of the root extract of *Rubia cordifolia linn* might be attributed to high antioxidant and anti-inflammatory capacities (33).

AQs such as 1_hydroxy_2_methylantraquinone possess good anti-oxidant activity. *Rubia cordifolia linn* extract is a source of AQs and was effective against renal damage and nephrotoxicity induced by cancer chemotherapeutic agents (34).

It was reported that ethanolic (EtOH) extracts of roots of *Rubia cordifolia linn* decreased LPO and increased the activity of SOD, CAT and GSH levels in renal tissue and prevented destructive effects of lead because of scavenging free radicals (35).

Toxicity

Rubia cordifolia Linn is a source of AQs such as nordamnacanthal. It was reported that this AQ might potentiate the cytotoxicity of drugs such as tamoxifen via four important mechanisms: 1) induction of apoptosis, 2) G_0/G_1-phase arrest, 3) fall in the mitochondrial membrane potential and 4) ROS

formation (36). Some evidences have examined the lethal dose (LD50) of *Rubia cordifolia Linn* that varied between 2000 and 3000 mg/kg (37, 38).

Roots extraction of *Rubia tinctorum* is known as madder color which is used as a food colorant. Madder color contains AQs such as lucidin, alizarin and their primeverosides such as lucidin_3_0_primeveroside (Lup) and ruberythric acid. Rubiadin (Rub) and lucidin are two main metabolites of Lup. There is evidence indicating an association between MC consumption and liver as well as kidney cancer (39). MC possesses carcinogenic effects in the kidney through increase in renal 8_0HdG levels. Surprisingly, this effect is opposed to RA_X II which was claimed to reduce the 8_0HdG (32).

Alizarin and lucidin exert cortical tubular degeneration. Lup and lucidin might induce renal cancer by increasing oxidative stress and the formation of DNA adducts (40). Karyomegaly (cell nucleus enlargement) of tubular cells and putative pre-neoplastic lesions are produced by Rub. Alizarin may also induce kidney cancer by increasing 8_OHdG levels in renal cells and cell proliferation in the proximal tubular cells of the outer medulla. Alizarin seems to be a nongenotoxic carcinogen. Oxidative stress induced by Lup may also play a limited role in carcinogenesis (39, 40). Luc might be a genotoxic carcinogen. It forms Luc-N(2)-dG and Luc-N(6)-dA adduct via sulfotransferase metabolic pathways (41).

Gastrointestinal Effects

Cold restraint-induced stress leads to gastric ulcers. Alcoholic extract of roots of *Rubia cordifolia linn* inhibited gastric ulcers induced by cold restraint-induced stress (42).

Inhibitory effects of oleananes on invasion and migration and of gastric cancer cell have been previously studied (43). Oleananes and other types of triterpenoids have been mainly isolated from the MeOH extract of roots of *Rubia cordifolia linn*. Pentacyclic triterpenoids are responsible for anti-inflammatory and mucus secretion stimulating effects. Triterpenoids are considered as good antioxidants. It has been indicated that Rubia implicate gastroprotective effects through reducing proteins and pepsin content and acidity of gastric fluid as well as increasing mucin content.

Gastroprotective effects of Rubia occur via two mechanisms:

1) Elevating prostaglandin synthesis and gastric mucus as well as decreasing pepsin and acid secretion. Incrteased hexose, sialic acid and other mucopolysacaride lead to increased total carbohydrates and glycoprotein contents of mucin

2) Increasing antioxidant defense molecules such as reduced glutathione, superoxide dismutase and catalase which suppress lipid peroxidation and other type of oxidative stress related complications (37, 44).

Rubia extract also exerts an antidiarrheal effect which seems to be related to phenolic acid and tannins compounds. Surprisingly, antipsychotic activities of Rubia extract have been reported which can cause constipation by inhibiting cholinergic and dopaminergic transitions and decrease the secretion of gastric and motility (45). Although the antidiarrheal effect of Rubia extract may be attributed to its antipsychotic activity, it could be via two major mechanisms:

1) Previous studies have revealed that oxidative stress promote lipid peroxidation and has a fundamental role in diarrheal pathogeneses. Anti-inflammatory and anti-oxidative properties of Rubia are due to decreases in malondialdehyde, myeloperoxidase as well as inflammatory cytokines such as interleukin_1β and TNF-α.

2) Antisecretory activity of Rubia results in its antimotility effects by reducing fluid accumulation (reduction both sodium and potassium excretion) in the intestinal lumen (45-49).

Lung Protective Effects

AQ alizarin is mainly extracted from the roots of *Rubia tinctorum*. Alizarin is used as an anti-oxidant in food industries (50). AQs such as emodin (3-methyl-1,6,8 thri hydroxy anthraquinone) could be extracted from *Rubia tinctorum* and other organisms such as algae, fungus, and bacteria (51). The protective effect of emodin against lung cancer has been reported. Alizarin reveals chemoprotective activity against nitrosodiethylamine (NDE) induced lung tumors by scavenging free radicals which contribute to lipid peroxidation and also by increasing antioxidant defense molecules such as GST and GSH (52). Emodin acts as an inducer of CYP 450 1A1 and 1B1 in human lung adenocarcinoma cells (53). Pulmonary inflammation could be alleviated by emodin and suppressing

mTOR/HIF-1α/ VEGF signaling pathway by the

Table 1: An overview of the effects of Rubia species on various organ system

Effect on:	Species	Compositions	Protective effect	Toxicity	Ref.
cancer	*Rubia cordifolia Linn* *Rubia akane nakai* *Rubia tinctorum L.*	RA-I, RA-XII, RA-XXIII RAXXIV, RA-XXV and RA-XXVI	inhibition of protein synthesis and induction of G2 arrest		(27-31)
	Rubia tinctorum L	Purpurin	selective activity on cell adhesion, proliferation, and migration of tumor		5
kidney	*Rubia yunnanensis*	RA-XII	Increasing in ROS formation such as CAT and SOD as well as reduced GSH levels and decrease in MDA, blocking IκBα/NFκB and MAP Kinases modulated by hemeoxygenase (HO)-1 and Nrf2 pathway		*32*
	Rubia cordifolia linn	hydro-alcoholic root extract AQs (such as 1_hydroxy_2_methylantraquinone) ethanolic (EtOH) extract	Restoring the magnesium high antioxidant and anti-inflammatory capacity (reducing lipid peroxidation)		*33*
	Rubia tinctorum	Nordamnacanthal		1)induction of apoptosis, 2) G_0/G_1-phase arrest, 3) fall in the mitochondrial membrane potential and 4) ROS formation	*(36)*
		Rubiadin (Rub), lucidin, Lup and Alizarin		increasing in renal 8_0HdG levels increasing oxidative stress and formation of DNA adducts	*32*
Gastrointestine	*Rubia cordifolia linn*	Oleananes and other types of triterpenoids	Elevating prostaglandin synthesis and gastric mucus, decreasing in pepsin and acid secretion, Increasing in antioxidant defense molecules		43

		phenolic acid and tannins compounds	constipation by inhibiting cholinergic and dopaminergic transition and decrease secretion of gastric and motility	45
Lung	*Rubia tinctorum*	Emodin	increasing GST and GSH, inducer of CYP 450 1A1 and 1B1, suppressing mTOR/HIF-1α/ VEGF signaling pathway	(51-54)
Skin	*Rubia cordifolia linn*	ethanolic extract ethyl acetate	anti-proliferative effects on keratinocytes	59
	Morinda citrifolia Linn	phytol , deacetyl asperuloside, quercetin	inhibits UV-induced erythema as a histamine H-1 receptor antagonists, antioxidant activity	61
	Mitracapus villosus		broad antifungal and antibacterial activities against Staph aureus and Candida albicans	62
Carbohydrate metabolism		tyrosinase, purpurin	A_glucosidase	57
Cardiovascular and Blood circulation	*Rubia cordifolia linn*	Alcoholic extract of roots	decreased hyperglycemia induced by alloxan, inducing VEGF receptor tyrosine kinase inhibitor II	42
	Rubia philippinensis	Arborinane triterpenoids	inhibited proliferation and migration of VSMCs due to the inhibition of PDGF	64
	Rubia yunnanensis	MeOH extraction of roots	antihyperlipidemic	65
	Rubiarbonone C		increasing the uptake of 2-deoxyglucose, improving insulin resistance and reduce cardiovascular complication in diabetic patients	65
	Rubia cordifolia Linn, Cymbopogon jwarancusa, Asparagus officinalis, Tribulus Terrestris, Echinops echinatus, Portulaca oleracea	methanolic extracts of roots	anti-NTPDase activity	66
Rheumatoid arthritis	*Rubia cordifolia Linn*	naphthohydroquinone mollugin	suppressing RANKL-signaling pathway	68

Bone resorption disease	*Rubia cordifolia Linn*	Physcion	reducing serum TRAP levels, inhibiting osteoclastogenesis		(68-70)
		Mollugin	preventing LPS-induced bone loss through the inhibition of osteoclast activity		68
		Alkaloids		antiproliferatIve, antimitotic and genotoxic effects at high doses and long term of use	(71,72)
	Rubia philippinensis	Xanthopurpurin, lucidin-ω-methyl ether		possess toxic effects towards breast cancer cells	8
	Rubie podantha	cyclopeptide rubipodanin B		NFκB inhibitory properties	73
CNS	*Rubia cardifolia Linn*	crystal of triterpene	Increasing of GABA and serotonin neurotransmitter substances		74
		Mollugin	increasing cellular viability against oxidative injury, inhibition of lipopolysaccharide		75
		ethanolic extract of root	inhibitory effects of the extract against acetylcholinesterase enzyme, monoamine Oxidase-A and free radical scavenging activity		76
		Alcoholic extract of roots and rhizomes	inhibiting neuropathic pain via GABA neurotransmitter and antioxidant mechanism, weak nootropic effect		42
	Rubia peregrina Linn	Tannins, flavonoids, glycosides	antioxidant activity and inhibited catalepsy and orofacial dyskinesia		(80,81)
Liver	*Rubia cardifolia*	H2O/MeOH extraction of roots	Decreasing serum levels of SGOT and SGPT		84
		Rubiadin	1) Decreasing in the elevated levels of SGOT, SGPT, serum alkaline phosphatase, and γ-glutamyl transpeptidase. 2) Reducing malondialdehyde 3) increasing glutathione S-transferase and glutathione reductase activities		85
		Dibutyl phthalate		1)Elevation of serum level of ALT 2)Leads to hypoglycemia and increase in total cholesterol. 3) change in total protein	7
	Rubia tinctorum L		declining the levels of ASAT, ALAT, ALP and GGT		90

	Gardenia gummifera	Decreasing AST, ALT, ALP in paracetamol induced liver damage		87
Rubiaceous herbs	Mollugin		decreasing CYP2E1 hydroxylation, inhibition of O-deethylation catalyzed by CYP1A2	(88,89)

expression of pro-inflammatory cytokine such as IL-6, IL-1β and TNF-α (54).

Effects on the Skin

Although contact dermatitis has been reported from Rubia (Castelain and Ducombs), Rubia species such as *Rubia cordifolia L*. have been used for skin disorders, including eczema, dermatitis skin ulcer, acne, wound, etc. (55).

The main AQs present in traditional remedy Pinda oil are alizarin, xanthopurpurin, purpurin and rubiadin. This oil is used for some dermatological disorders such as eczema, pruritus and cracked skin as well as some inflammatory diseases such as arthritis rheumatoid (56). Further studies are required to be conducted in order to determine the exact component responsible for these effects and the underlying mechanisms.

Multifunctional enzyme tyrosinase affects melanogenesis through the hydroxylation of L-tyrosine to L-Dopa and subsequent oxidation of L-Dopa to Dopachrome. Tyrosinase inhibitory potential of purpurin, alizarin and quanizarin has been reported previously. In addition to tyosinase, purpurin bot not alizarin and quanizarin, strongly inhibits acetylcholinesterase (57). Investigations have sown that purpurin reveals competitive suppression on the monophenolase function of the tyrosinase. Actually, purpurin persuades conformational changes in tyrosinase secondary structure due to the rearrengment of hydrogen binding network at the polypeptide chain of tyrosinase active site. Thus, tyrosinase may lose its catalytic action and tend to bind to L-Dopa. Consequently, *R.cordifolia* may be efficient in the treatment of hyperpigmentary diseases such as dark skin discoloration so called melasma (58).

Ethanolic extract of the root of *Rubia cordifolia linn* has exerted anti-proliferative effects on cultured keratinocytes, and its ethyl acetate fraction has a greater level of anti-proliferative effects than the crude ethanolic extract. Moreover, it displayed apoptogenic properties on human keratinocytes and promoted keratinocyte differentiation so that it could be applied in topical antipsoriatic formulations (59).

Along with antipsoriatic effects, alcoholic extract of Rubia in gel formulation has been applied as anti-acne as well as wound healing accelerator (55, 60).

Morinda citrifolia Linn leaves have antioxidant effects because of their compounds, including phytol and deacetyl asperuloside. Furthermore, existent quercetin in leaves of the plant inhibits UV-induced erythema as a histamine H-1 receptor antagonist. Moreover, previously studies have demonstrated the antioxidant activity of the leaves and the scavenging of free radicals that is another mechanism whereby the UV damage is relieved (61).

On the other hand, some assays have indicated that *Mitracapus villosus* is effective in the treatment of some skin disorders (including infectious dermatitis, eczema and scabies), and it has also been shown that *Mitracapus villosus* can display broad antifungal and antibacterial activities against standard strains and clinical bacterial isolates of *Staphylococcus aureus* and *Candida albicans* responsible for common skin infections (62).

Effects on Carbohydrates Metabolism

In addition to tyrosinase, purpurin strongly inhibits acetylcholinesterase and has a remarkable inhibitory effect on α-glucosidase (57). α-glucosidase inhibitors have been used in the treatment of type 2 diabetes mellitus. These findings have revealed the potential antidiabetic effects of Rubia species. It has been reported that *Rubia tinctorum* root extracts are effective in the management of type II diabetes mellitus. Among various solvents for extraction, methanolic and ethyl acetate extracts were more efficient than hexane and chloroform extracts (63).

Cardiovascular and Blood Circulation

Glucose analog alloxan is a toxic agent which increases blood sugar level. Alcoholic extract of the roots of *Rubia cordifolia linn* significantly decreased hyperglycemia induced by alloxan (42).

Vascular smooth muscle cells (VSMCs) are the particular type of smooth muscles found on the wall and media of blood vessels. Unusual VSMCs proliferation and migration lead to the development of cardiovascular dysfunctions such as atherosclerosis. Cytokines like TNF-α and growth factors such as platelet-derived growth factor (PDGF) could induce abnormal proliferation and migration of VSMCs. Arborinane triterpenoids found in the ethanolic extract of the roots of *Rubia philippinensis* inhibited the proliferation and migration of VSMCs due to the inhibition of PDGF (64).

Hyperlipidemia considerably leads to the development of cardiovascular diseases such as heart failure, stroke, and coronary artery disease, which are the most common causes of morbidity and mortality throughout the world. Traditionally, in Chinese medicine the roots of *Rubia yunnanensis Diels* (Rubiaceae) have been used in the treatment of various disorders, including cardiovascular and metabolic diseases. The antihyperlipidemic properties of chemicals isolated by the extraction of the roots of *Rubia yunnanensis* with MeOH have been reported in vitro and in vivo. Moreover, *Rubiarbonone C* remarkably raised the uptake of 2-deoxyglucose into the cells which could improve insulin resistance and reduce cardiovascular complication in diabetic patients (65).

Aggregation of platelet is a key factor in cardiovascular disease and ADP is one of the primary motivations of aggregation. Inhibitory enzyme nucleoside triphosphate diphosphohydrolase (NTPDase) has a significant role in the regulation and initiation of coagulation. Activity of NTPDase results in the formation of nucleoside monophosphate (AMP) via the hydrolysis of ATP and ADP. Among the methanolic extracts of about 70 plants, *Rubia cordifolia Linn, Cymbopogon jwarancusa, Asparagus officinalis, Tribulus Terrestris,* and *Echinops echinatus, Portulaca oleracea* exerted anti-NTPDase activity (66) so that aggregation of platelet could be affected by the use of Rubia roots extract. Nevertheless, some studies have reported anti-thrombotic properties of *Rubia cordifolia Linn* extract. As a traditional Chinese medicine, *Rubia cordifolia Linn* has been used in the treatment of diseases related to blood stasis syndrome (BSS) subject to its potential anti-thrombotic activities. *Rubia cordifolia Linn* root extract induces VEGF receptor tyrosine kinase inhibitor II which may lead to angiogenesis (67).

Joint and Bone

Rheumatoid Arthritis

RBC count and Hb level is reduced and ESR is increased in chronic rheumatoid arthritis. Ethanolic extract of Rubia *cordifolia* roots increase RBC count and Hb level and reduce ESR in chronic rheumatoid arthritis.

Some studies have indicated that Rubia improves this anaemic condition and makes these parameters close to normal levels.

On the other hand, elevating the levels of C-reactive protein, WBC count and Copper ion are indications for inflammatory diseases that can be significantly declined by Rubia (38).

The receptor activator of nuclear factor-κB (RANK) is the membrane receptor which is activated with RANK-Ligand (RANKL). Excessive activation of the RANK leads to progressive bone resorption and osteoporosis. Hence, downregulation of RANKL signaling pathway could be considered for the treatment of bone disorders such as osteoporosis. An active ingredient of *Rubia cordifolia Linn* naphthohydroquinone mollugin improved osteoporosis by suppressing RANKL-signaling pathway. Moreover, mollugin exhibited anti-cancer properties and improved blood circulation (68).

Bone Resorption Disease

In traditional medicine, roots of *Rubia cordifolia Linn* have proved to have beneficial effects on fractured bones. This could be attributed to the AQ physcion of the ethanolic root extract of Rubia which has osteoblastic activity and inhibits osteoclastogenesis. Tartrate-resistant acid phosphate (TRAP) is a biomarker of bone turnover. Ethanolic extracts of the roots of *Rubia cordifolia Linn* could reduce serum TRAP levels showing the inhibitory effect on bone resorption. There are two key cytokines generated largely by osteoblast and stromal cell, modulate osteoclastogenesis, macrophage colony-stimulating factor (M-CSF) and RANKL. M_CSF (or CSF-1) plays

a role in osteoclast differentiation by modulating proliferation and survival of osteoclast precursor cell. It has been exhibited that the lack of functional M-CSF expression may lead to osteoporosis. In fact, monocytes macrophage cells in the presence of M-CSF and RANKL differentiate into mature bone resorbing osteoclasts. RANKL binding to RANK results in the activation of NF_KB, protein kinase B, TNF receptor associated factor 6. Subsequently, MAP kinase pathways are activated. This pathway finally leads to the expression of different genes such as TRAP. Furthermore, mollugin prevents LPS-induced bone loss through the inhibition of osteoclast activity. Consequently, mollugin reveals trapeutic potential as an anti-osteoclastogenesis (68-70).

Toxic Effects

Chromosal aberrations and mitotic index have been considered for measuring cytogenetic effects of chemicals. Alkaloids extracted from the roots of *Rubia cordifolia Linn* induce chromosomal aberration and exert antiproliferative, antimitotic and genotoxic effects at high doses and in long-term use. Marked reduction in mitotic index has been reported by the use of alkaloids which could be due to either decline in the number of cells moving from G2 into prophase or inhibition of DNA, RNA and protein synthesis. Hence, alkaloids could be considered as strong mitotic inhibitors and anti-proliferative agents (71, 72). Xanthopurpurin and lucidin-ω-methyl ether from dichloromethane fraction of the root of *Rubia philippinensis* have toxic effects towards breast cancer cells. These molecules could be considered as new lead compounds in cancer treatment (8). Moreover, cytotoxic cyclopeptide rubipodanin B isolated by the extraction of roots of *Rubie podantha* with MeOH indicated NF-κB inhibitory properties (73).

Central Nervous System (CNS)

The anticonvulsant effects of crystal of triterpene isolated from the acetone fraction of petroleum ether extract of *Rubia cardifolia Linn* have been examined. Crystal of triterpene dose dependently inhibited the incidence of convulsions induced by electrical kindling, maximum electroshock as well as strychnine and pentylenetetrazole. It also had inhibitory effects on convulsions induced by electrical kindling and lithium-pilocarpine administration. Crystal of triterpene could not affect seizures induced by strychnine. GABA and serotonin neurotransmitter substances were raised by the triterpene that proves its anticonvulsant property (74).

Inflammation and oxidative stress are two potential factors related to neurodegenerative diseases. Mollugin exerted significant anti-oxidative and anti-inflammatory effects. It also increased cellular viability against oxidative injury caused by glutamate-induced cytotoxicity and inhibited lipopolysaccharide induced pro-inflammatory agents. These effects could be related to the expression of Heme Oxygenase-1through the p38 kinase and Nrf2 pathways (75).

Alzheimer's disease symptoms are associated with the accumulation of amyloid-beta and tau protein in CNS. β_amyloid injection through intra cerebroventricular induces cognitive dysfunction which is inhibited by the administration of the ethanolic extract of the root of *Rubia cardifolia Linn.* These effects could be related to the inhibitory effects of the extract against acetylcholinesterase enzyme, monoamine Oxidase-A and free radical scavenging activity (76).

Oxidative stress leads to many pathophysiological disorders in the body that start with free radicals and cause apoptosis, necrosis, and cell death which could initiate neurodegenerative disorder in CNS. Extracts of the roots of Rubia species represented potential antioxidant activity, by repairing glutathione peroxidase activity, decreasing iNOS expression and also by increasing the antioxidant gene Cu-Zn superoxide dismutase (77).

Potential neuroprotective and antioxidant activities of chemicals isolated by the extraction of the roots of *R. cordifolia* with MeOH were studied on orofacial dyskinesia induced by reserpine administration. AQs are the main components of this plant that shows anti-oxidative stress activity through increasing the levels of superoxide dismutase, catalase, glutathione reductase, and inhibition of Lipid peroxidation. Tardive dyskinesia (TD) is caused by reserpine administration with the symptom of orofacial dyskinesia characterized by vacuous chewing movements, tongue protrusion and orofacial bursts. Extraction of the roots of Rubia with MeOH remarkably inhibited orofacial dyskinesia and catalepsy induced by reserpine (78).

Neuropathic pain can be induced by nerve damage which arouse from nociceptive receptor and descending modulatory pathways in the CNS. Paclitaxel induces

neurotoxicity that leads to neuropathic pain manifested by symptoms such as allodynia and hyperalgesia. Alcoholic extract of the roots and rhizomes of *Rubia cardifolia Linn* has prophylactic and preventive effects on neuropathic pain induced by paclitaxel in a dose-dependent manner. The extract also potentially inhibits neuropathic pain via GABA neurotransmitter and antioxidant mechanism (79).

Rubia peregrina Linn is considered to be traditionally useful as an aphrodisiacand diuretic. Tannins, flavonoids, glycosides, and steroids have been isolated by the extraction of the aerial parts of *R. peregrina L* with EtOH. Chemicals isolated by the extraction of the aerial parts of *R. peregrina L* with EtOH exhibited significant antioxidant activity and inhibited catalepsy and orofacial dyskinesia induced respectively by haloperidol and reserpine (80).

Many developmental diseases in CNS including emotion, learning, and memory are related to neurotransmitters such as dopamine, serotonin, norepinephrine and epinephrine. Some of these deleterious conditions are related to adverse drug reactions. Chemicals isolated by the extraction of the aerial parts of *R. peregrina L* with EtOH inhibited catalepsy mediated by anti-dopaminergic haloperidol and head twitches induced by lithium which is related to serotonine (81). Along with antioxidant activity, chemicals isolated by the extraction of the roots of *R. cordifolia* with MeOH have anticholinergic effects (82). As a result, the beneficial effects of the root extract of Rubia against anti-dopaminergic drugs' side effect can be attributed to its anticholinergic activity.

Neuropharmacological investigation of chemicals isolated by the extraction of the roots of *R. cordifolia* with EtOH revealed that its use is associated with CNS depression and skeletal muscle relaxation. The extract significantly diminished motor coordination response and alertness (83).

Alcoholic extract of the roots of *Rubia cordifolia Linn* increased GABA in the brain. Moreover, the extract has weak nootropic effects (42).

Effects on the Liver

Hepatoprotective Activity

Exposure of the liver to paracetamol and carbon tetrachloride (CCI4) can cause hepatic damage which is characterized by increased serum levels of glutamic oxaloacetic transaminase (SGOT) and glutamate pyruvate transaminase (SGPT). Chemicals isolated by the extraction of the roots of *Rubie cordifolia* with H2O/MeOH decreased serum levels of SGOT and SGPT induced by paracetamol and CCl4 (84).

Moreover, CCl4 induced elevation in the serum level of hepatic enzymes that had been prevented by rubiadin. Rubiadin showed dose-dependent hepatoprotective activity by:

1) Decreasing the elevated levels of SGOT, SGPT, serum alkaline phosphatase, and γ-glutamyl transpeptidase,

2) Reducing malondialdehyde

3) Increasing glutathione S-transferase and glutathione reductase activities (85).

The well-known indigenous medicine in India, *Gardenia gummifera*,, showed hepatoprotective effects against hepatotoxicity-induced paracetamol. *Gardenia gummifera* metanolic extract decreased the levels of marker enzymes (AST, ALT, ALP) in paracetamol-induced liver damage in rats. This effect may be caused by the stabilization of plasma membrane and repair of hepatic tissue damage induced by paracetamol (86). Moreover, *Gardenia gummifera L.f. fruit methanol* extract has no mortality and weight loss at the dose of 2000 mg/kg (87).

Toxic Effects

The liver is the most significant detoxifying organ in our body. Important metabolisms like conversion of the nutrients and toxic substances into harmless substances occur in it. The most considerable liver enzymes involved in metabolism are cytochromes P450 (CYPs). The study of activity of hepatic CYP is important for predicting drug-drug interactions and awareness of undesirable adverse effects. Mollugin isolated from Rubiaceous herbs significantly decreased CYP2E1 hydroxylation (88). Moreover, mollugin strongly inhibited O-deethylation catalyzed by CYP1A2 (89). These inhibitory effects should be considered as potential herb-drug interactions.

Recently, Fatimazahra Marhoume *et al.*, have reported that *Rubia tinctorum's* extract can protect hepatic cell by meaningfully declining the levels of cytosolic enzymes activities, including ASAT, ALAT, ALP and GGT induced by CCl_4 treatment rats. Consequently, *Rubia tinctorum L* may have a protective effect against liver damages induced by CCl_4 (90).

Phthalates are known as endocrine-disrupting agents,

and dibutyl phthalate is the most widely synthesized derivative which is used as a plasticizer (91). Dibutyl phthalate isolated by the extraction of fruits of *R. cordifolia* with EtOH can cause the following:

1) Elevation of the serum level of ALT showing liver damage,

2) Change in lipid and carbohydrate metabolism leading to hypoglycemia and increase in total cholesterol,

3) Change in total protein due to the hepatocellular damage (7).

An overview of the effects of Rubia species on various organs investigated in this review has been shown in Table 1.

Conclusion

Rubia extracts have been applied for skin care and urinary tract disorders. They can have anticancer, anti-inflammatory, antioxidant, antimicrobial, antidiabetic, anti-arthritic, antiseizure and spasmolytic activities, and can be good candidates for clinical examination in these viewpoints.

Rubia species produce phytoalexins – AQs and Rubiaceae type cyclopeptides (RAs) such as RA-XXIII, RA-XXIV, and RA-VII from the methanolic extract of the roots of Rubia that can have cytotoxic effects and anticancer properties. Hence, it is a rational approach to develop plant cells cultivation techniques aimed to increase the synthesis of AQs and RAs in Rubia plants on an industrial scale. The anticancer property of RA-VII has been tested in vitro and in clinical trials. Clinical observations on *Rubia cordifolia linn* showed an increase in leukocytes count in leucopenia.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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