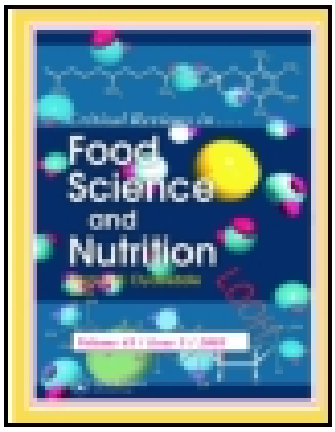

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Therapeutic and Nutraceutical Potential of Rosmarinic Acid - Cytoprotective Properties and Pharmacokinetic Profile

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Review article

Therapeutic and nutraceutical potential of rosmarinic acid - cytoprotective properties and pharmacokinetic profile

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Abstract

Rosmarinic acid (RA) is a natural polyphenolic antioxidant derived from many common herbal plants. This compound displays several important biological properties, including anti-inflammatory, antiviral, antibacterial, antidepressant, anticarcionogenic, and chemopreventive properties. The importance of its activities and its possible application in processed foods as a natural antioxidant has been reached a new interest levels in recent years. The health effects of this polyphenol depend greatly on both its intakes and bioavailability. This review focuses on the importance of RA as a dietary supplement, and summarizes its pharmacokinetics and metabolism, including the factors that limit its oral bioavailability which leads to a lower therapeutic action. Further experimental investigations are needed to optimize and enhance the oral bioavailability of this natural compound which consequently will help increasing therapeutic efficacy of RA *in vivo*.

Keywords: Rosmarinic acid, therapeutic, nutraceutical, cytoprotective properties, pharmacokinetic profile, *in vivo* models.

1. Introduction

In the last few years, an important area where food nanotechnology is increasingly used is the design and development of new functional and nutraceutical products from natural sources in order to improve food quality, without the need of synthetic additives, as well as to develop novel functionalities, especially related to health promotion, such as antioxidant, anti-inflammatory, and anticarcinogenic properties (Weiss et al., 2006; Shahidi, 2009; Neethirajan and Jayas, 2011). There is a growing interest and renewed research on the possibility of application of polyphenols in functional foods, nutraceutical and pharmaceutical industries (Shen et al., 2014; Grace et al., 2013; Kalim et al., 2010). Polyphenolic phytochemicals are commonly an integral part of the daily human diet presented in foods and beverages which are widely assumed as supplements with preventive and therapeutic potential for diseases (Tyagi et al., 2010; Gonzalez-Vallinas et al., 2013) .

The effectiveness of polyphenols depends on preserving their stability, their respective intakes and the bioavailability of the active ingredients. In this context, the development of strategies to improve the oral bioavailability is crucial for the development of more therapeutic efficacious compounds. To this end, it is crucial to clearly elucidate the pharmacokinetic profile which will be the key to determine the precise mechanisms of action.

Among the polyphenolic substances, rosmarinic acid (RA), a naturally occurring plant compound, has attracted considerable interest due to their wide important therapeutic properties, and health benefits. This article firstly summarizes the protective biological activities already reported for RA and then critically reviews the most relevant animal and human studies that

focused on the elucidation of RA oral bioavailability and pharmacokinetic data and highlights future prospective in this research field.

2. Rosmarinic acid as a nutraceutical

RA is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. This hydrophilic polyphenolic compound is widely found in the plant kingdom, mainly in medicinal herb plants. RA is found throughout the *Boraginaceae*, whereas within the *Lamiaceae* it is restricted to the sub-family *Nepetoideae*. Examples include ferns of the family *Blechnaceae*, lower plants such as the hornworts and in monocotyledonous plants like the sea grass family *Zosteraceae*, the related *Potamogetonaceae* as well as the *Cannaceae* (Park et al., 2008). Examples of herbs from the *Lamiaceae* family and their concentrations in RA are depicted in Table 1. The profile of a plant, in terms of its many constituents that possess antioxidant features, may vary according to the geographical area of growth, soil, climatic conditions, harvest period and processing conditions afterwards (Dweck, 2009). Furthermore, the storage conditions of the plant, the part of the plant considered, the extraction time and temperature, and the solvents used in that extraction will all also have a significant implication upon the final chemical composition of extracts for human ingestion (Dweck, 2009).

This polyphenol has a number of potential biological activities, summarized in Table 2. The health promoting effects reported by RA are: antioxidant, anti-inflammatory, antibacterial, anti-angiogenic, anti-mutagenic, antidepressant and antiallergenic effects (Furtado et al., 2008; Petersen and Simmonds, 2003; Swarup et al., 2007). The pharmacological properties are thought

to be based on the enhancement of superoxide and hydroxyl scavenging, inhibition of both low-density lipoprotein and oil oxidation, inhibition of haemolysis and hyaluronidase, and β-hexosaminidase activities (Baba et al., 2004). Activities and potential mechanisms of action will be detailed in the following sub-sections.

2.1 Antioxidant activities

Some experiments have reported the strong capacity of RA to exhibit antioxidant activity in the biological systems, through the scavenging of reactive nitrogen species, peroxynitrite and various other reactive oxygen species (ROS) (Choi et al., 2002; Qiao et al., 2005). RA has been shown to effectively scavenge 2,2-diphenyl-1-picryhydrazyl, a molecule used to determine the intrinsic free radical scavenging activity (Alamed et al., 2009), which denotes its high antioxidant activity. *In vivo* studies using mouse models of Alzheimer's disease and amyotrophic lateral sclerosis have shown that RA significantly alleviates memory impairment associated with amyloid beta protein neurotoxicity and significantly delays disease onset and prolongs lifespan in the G93A mutant SOD1 mouse model, respectively (Alkam et al., 2007; Shimojo et al., 2010).

2.2 Antimicrobial properties

Moreno et al. (2006) investigated *Rosmarinus officinalis* extracts to identify their bioactive constituents and properties, as well as the distribution and levels of antioxidants. They found a strong correlation between antioxidant activity and total phenolic content, with all rosemary extracts displaying significant radical scavenging activity. A methanol extract that contained

30% carnosic acid, 16% carnosol and 5% RA was substantially more effective as antimicrobial agent against Gram-positive bacteria, Gram-negative bacteria, and yeast than was a water extract containing 15% RA. The investigators concluded that the antimicrobial activity of rosemary extracts was linked to their phenolic composition, and that carnosic acid and RA are probably the primary antimicrobial components of rosemary.

2.3 Anti-inflammatory properties: focus on hepatoprotective activity

Rosmarinic acid was identified as an anti-inflammatory agent. The anti-inflammatory properties are promoted by the inhibition of lipoxygenases and cyclooxygenases and the interference with the complement cascade. The compound also has a strong inhibitory action on cellular pathways and on the activity of pro-inflammatory cytokines (Petersen and Simmonds, 2003; Ritschel et al., 1989).

In 2004, Osakabe et al. (2004a) reported that oral supplementation with RA was an effective treatment for seasonal allergic rhinoconjunctivitis patients, due to inhibition of the inflammatory response and the scavenging of ROS.

RA was suggested to also exhibits hepatoprotective activities (Osakabe et al., 2002; Li et al., 2010). In an *in vivo* study, RA increased serum albumin/globulin, decreased serum levels of hyaluronic acid, laminin and collagen types III, and content of hydroxyl proline in fibrotic liver, decreased hepatic fibrosis severity, as well as ameliorated liver histological morphology, reduced transforming growth factor-beta1 and connective transforming growth factor expression of fibrotic liver. These findings suggest that RA has potentially anti-fibrogenic effects and can be a promising candidate for ameliorating liver fibrosis (Li et al., 2010).

Osakable et al. (2002) reported that RA (135 mg/kg of RA, an equivalent dose to 200 mg/kg of Perilla Extract (PE)) was able to reduce liver injury induced by lipopolysaccharide in D-galactosamine-sensitized mice, due to the scavenging of superoxide molecules and inhibition of peroxinitrite formation induced by inducible nitric oxide synthase. Similarly, Sanbongi et al. (2003) observed that RA inhibited the formation of nitrotyrosine induced by diesel exhaust particles, as well as neutrophilic inflammation and edema in the lung, in mice treated for 3 days with RA at a dosage of 2 mg/100µL/day, suggesting a reduction of pro-inflammatory molecule expression and enhancement of antioxidant activity as key protective effects.

2.4 Anti-cancer properties

RA is considered to possess cancer chemopreventive properties. Oral administration of RA prevented the formation of skin tumors during 7,12-dimethylbenz(a)anthracene (DMBA) induced mouse skin carcinogenesis (Sharmila and Manoharan, 2012). In addition, oral administration of RA (100 mg/kg) brought back the status of phase I and phase II detoxification agents, lipid peroxidation by products, antioxidants and apoptotic markers (p53, Bcl-2, caspase-3 and caspase-9) in DMBA-treated mice. The results of another study suggest that RA (100 mg/kg) suppresses oral carcinogenesis by stimulating the activities of detoxification enzymes, improves the status of lipid peroxidation and antioxidants, and downregulates the expression of p53 and bcl-2 during DMBA-induced oral carcinogenesis (Anusuya and Manoharan, 2011).

Osakabe et al. (2004b) also reported on the effects of a RA–rich PE in a two-stage murine skin cancer model. They also showed a significant suppression of tumorigenesis as a result of the topical application of 2 mg/mouse of the extract following tumor initiation with DMBA. Tumor promotion was achieved by the use of 12-tetradecanoylphorbol 13-acetate (TPA). The

researchers noted that anti-inflammatory activity 5 hours after TPA treatment was equivalent between a PE containing 68% RA and a commercially available RA. In addition, TPA-induced increases in myeloperoxidase activity, as well as the production of certain chemokines, were significantly reduced by pretreatment with PE or RA, as were expression levels of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 mRNA. Furthermore, pretreatment with the extract or with commercial RA significantly reduced reactive oxygen radical synthesis (of 8-hydroxy-2-de-oxyguanosine) induced by double treatment of TPA. The researchers attributed part of the anticarcinogenic activity of PE to the independent mechanisms associated with RA, free radical scavenging capacity and inflammatory response suppression.

In addition, Furtado et al. (2008) reported the antimutagenic effect of RA using an *in vivo* mouse peripheral blood micronucleus assay. The compound exhibited potential to inhibit the mutagenicity induced by doxorubicin (DXR) in peripheral blood cells. DXR was reported to form superoxide anions that ultimately generate $^{\cdot}OH$ radicals and induce the number of micronuclei in the blood cells due to mutations. The RA also exerted an anti-mutagenic potential by scavenging these hydroxyl radicals.

2.5 Cardioprotective properties

Although the reports are scarce, there are also some evidences of the importance of RA on the cardiovascular system. Kim et al. (2005) investigated the inhibitory effect of RA (20 µg/mL) on adriamycin (ADR)-induced apoptosis in H9c2 cardiac muscle cells at a mechanistic level. *In vitro*, ADR significantly decreased the viability of H9c2 cells, and this was accompanied by apoptotic features, such as a change in nuclear morphology and caspase protease activation. RA

was found to inhibit these apoptotic characteristics by reducing intracellular ROS generation and by recovering the mitochondria membrane potential. Results of this exhaustive study suggested that RA can inhibit ADR-induced apoptosis, suggesting that RA should be viewed as a potential chemotherapeutic agent that inhibits cardiotoxicity in ADR-exposed patients. In addition, another study was undertaken to investigate whether RA supplementation prevented cardiac abnormalities and hypertension in fructose-fed rats (FFR), as the RA has insulin-sensitizing and antioxidant effects in high fructose-fed model of insulin resistance (Karthik et al., 2011). RA supplementation to FFR significantly improved insulin sensitivity, reduced lipid levels, reduced oxidative damage, and the expression of p22phox subunit of nicotinamide adenine dinucleotide phosphate reduced oxidase, and prevented cardiac hypertrophy. The blood pressure was also lowered by RA through a decrease in endothelin-1 and angiotensin-converting enzyme activity and an increase in nitric oxide levels. Histology revealed a decrease in myocardial damage, in RA-supplemented FFR. From these findings, it was suggested that RA acts as a vasoactive substance and as a cardioprotector through its antioxidant effects. Thus, RA may be useful in reducing the cardiovascular risk associated with insulin resistance (Karthik et al., 2011).

3. Pharmacokinetic profile of rosmarinic acid

The pharmacokinetics, including tissue distribution, metabolism and excretion of the RA in target organs and their metabolic fate in serum, remain to be fully elucidated. However, several studies, mainly in animal models, have studied the pharmacokinetic profile of RA, as summarized in Table 3 and Figure 1.

The pharmacokinetic parameters of RA in Sprague Dawley rats following intravenous administration of 60 mg/kg S. miltiorriza depside salts were evaluated by Li et al. (2007). The concentration-time curves were adequately described by a two-compartment model, and the resulting pharmacokinetics parameters were analyzed. In brief, the reported elimination half-life for RA was 0.75h; AUC $_{(0\text{-}6h)}$ = 6.6 mg.h/L, mean residence time$_{(0\text{-}\infty)}$ 0.32h, $T_{1/2}$ = 0.12h and clearance =1.02 L/h/kg (Table 3). Additionally, the AUC value of RA in kidney was much higher than those in other tissues; RA was found to be eliminated mainly via kidney into urine, and a low value of AUC was found in the brain, suggesting that RA does not efficiently cross the blood-brain barrier.

Lai et al. (2011) also studied the pharmacokinetics of RA in rats following intravenous and oral administrations. They demonstrated a more rapid distribution and elimination from systemic circulation with a T1/2,λZ of 56.45 min after intravenous administration (Table 3). After being administered orally, RA was absorbed and eliminated more rapidly, with a $Tmax_1$ of 10min, a $Tmax_2$ of 45 min and a T1/2,λZ of 63.68 min.

Konishi et al. (2005) orally administered RA (100 µmol/kg) in rats to investigate their intestinal absorption. RA in portal vein peaked at 10 min after administration, with a Cmax of 1.36 µmol/L for RA; AUC for intact RA in portal vein was calculated from the serum concentration-time profile (60.4 µmol.min/L).

In vivo studies were performed to understand the metabolism and bioavailability of RA. Rosmarinic acid is well absorbed from gastrointestinal tract and from the skin. By topical administration of RA to the skin of rats, RA was percutaneously absorbed and distributed in blood, skin, bone and muscle, while intravenous administration in these animals led to RA being

detectable in various tissues, such as lung, spleen, heart and liver (Ritschel et al., 1989). Also, in rats orally administered with RA, the polyphenol was absorbed, degraded, and/or conjugated as m-hydroxyphenylpropionic acid, m-coumaric acid, and sulphated forms of caffeic acid and ferulic acid (Baba et al., 2004), which were then absorbed by a specific intestinal monocarboxylic acid transporter-mediated active process (Konishi and Kobayashi, 2005), before being excreted gradually in the urine (Baba et al., 2004) (Table 3). These results show that, in the rat, RA may be absorbed from the digestive tract and skin, and is then distributed in various tissues. In contrast, Nakazawa and Ohsawa (2000) demonstrated that intake of PE in humans resulted in a β-glucuronic metabolite, found in the urine, suggesting that this metabolite was derived from RA contained in the PE. This finding indicates differences in the metabolism of RA between humans and rats, most probably due to differences in the location of enzymes, affinity of substances and characteristics of the enzymatic reactions.

In a human study, Baba et al. (2005) proposed a hypothetical metabolism for RA based on the action of microbial esterase in the digestive tract, hydrolyzing the ester linkage in RA. In this study, they demonstrated that RA contained in PE was absorbed, conjugated, and/or methylated in tissues such as the digestive tract and liver, with a small portion of RA being degraded into various components, such as glucuronidated or sulphated conjugates of caffeic acid, ferulic acid and a trace of m-coumaric acid, being then rapidly excreted in urine. Other studies have shown that gut bacterial esterases are able to hydrolyze the ester bond in hydroxycinnamates (Couteau et al., 2001; Gonthier et al., 2006). These enzymes could play an important role in the uptake of RA, justifying the potential health benefits of this class of molecules and their action could be an interesting approach for applications in food products.

Polyphenols that are not absorbed (90–95% of the total polyphenol intake) are transported to the colon where they can be readily hydrolyzed by colonic microflora to simpler chemical compounds. During the course of absorption, polyphenols undergo other structural modifications due to the conjugation process that takes place in the small intestine and, mostly, in the liver (Felgines et al., 2005). The conjugation, that includes methylation, sulfation, and glucuronidation, represents a metabolic detoxication process common in many xenobiotic compounds that restricts the potential toxic effects and facilitates biliary and urinary elimination by increasing their hydrophilicity.

It has been noted that the extent of metabolism may vary considerably, due to differences in microflora and food matrices (Van Duynhoven et al., 2011) .Clostridium and Eubacterium are the main genera involved in the metabolism of many phenolics such as isoflavones (daidzein), flavonols (quercetin and kaempferol), flavones (naringenin and ixoxanthumol) and flavanols (catechin and quercetin) (Selma et al., 2009), but individuals may have different and/or altered flora profiles, which may affect their metabolism (Van Nuenen et al., 2004).

The colonic metabolism of rosmarinic acid is scarcely reported. However, Bel-Rhlid et al. (2009) investigated the in vitro hydrolysis of RA with different esterases and with a probiotic bacterium Lactobacillus johnsonii NCC 533 (La1) isolated from the human intestinal microbiota. Among tested enzymes, only chlorogenate esterase was able to hydrolyze RA, confirming the hypothesis that RA could be cleaved by intracellular cinnamoyl esterases, which have been found in the gut microbiota of animals and humans (Couteau et al., 2001). Complete hydrolysis of RA was achieved when La1 was added to rosemary extract in the gastrointestinal tract model (GI model), while, in turn, no hydrolysis of RA was observed after the passage of rosemary extract through

the GI model without addition of La1. Thus, RA is hydrolyzed neither chemically under the conditions of the GI model (temperature, pH, and bile salts) nor by secreted enzymatic activity (lipase and pancreatic enzymes). The study supports the hypothesis that RA is degraded by enzymes or gut microflora before their absorption and metabolization.

The polyphenol microflora transformation was proposed by animal or human studies (Plumb et al., 1999; Rechner et al., 2002; Williamson et al., 2000). Gut microflora hydrolysis is often thought to benefit bioavailability of phenolic compound including flavonoid glycoside. When sugar unit is removed, the resulting aglycone can be absorbed more readily. For example, rutin, hesperidin, naringin, baicalin, puerarin, daidzin and poncirin were hydrolyzed to their respective aglycones by human intestinal microflora, and the resulting aglycones were absorbed better (Kim et al., 1998). However, in some cases the parent compounds (e.g., glycosides) are the biological active sources and when hydrolysis occurs the bioavailability will be decreased; for example, the ester bond of rosmarinic acid was hydrolyzed by human microflora to form two simple less active phenolic acids (Konishi and Kobayashi, 2005).

Bioavailability of each polyphenol differs, however there is no relation between the quantity of polyphenols in food and their bioavailability in human body. Polyphenol bioavailability depends on several other factors. The physicochemical characteristics of polyphenols appear to dictate absorption and metabolism and are determined primarily by chemical structure (Bravo, 1998). Relatively small molecular weight phenolic acids, such as gallic acid and caffeic acid, are easily and well-absorbed through the gut followed by catechins, flavanones, and quercetin glucosides, while larger molecular weight polyphenols, such as proanthocyanidins, galloylated tea catechins and the anthocyanins, are very poorly absorbed.

According to the route of administration, the efficiency of these compounds depends on their bioavailability and integrity. Indeed, only a small proportion of molecules administered orally are absorbed, because of insufficient gastric residence time, first pass effect, low permeability and/or low solubility (Amidon et al., 1995). Their instability during food processing, distribution or storage, as well as the food matrix composition are other factors that limit the activity and the potential health benefits of polyphenols (Manach et al., 2004) (Fig. 1).

Different studies have been carried out to evaluate the interaction between polyphenols and food matrices, such as milk (Van Het Hof et al., 1998; Hollman, 2001), olive oil (Shishikura et al., 2006) or sugar (Schramm et al., 2003). The evidence suggests that variations in polyphenol absorption also occur due to interactions between polyphenols and other food components, such as proteins, which destabilize these compounds and decrease their bioaccessibility and subsequent bioavailability after being ingested (Serafini et al., 2009). In this context, bioaccessibility is important to consider since it reflects the actual fraction of polyphenols that is released from the food matrix during digestion and thus becomes available for intestinal absorption. For example, *in vivo* tests showed that ingestion of milk with epicathechin led to differences in metabolite excretion profiles (Roura et al., 2008); furthermore, the ingestion of blueberries in association with milk impaired the *in vivo* antioxidant properties of blueberries and reduced the absorption of caffeic and ferulic acids (Serafini et al., 2009). The interactions of cathechins, for example, are suggested to occur with proline rich proteins (e.g. caseins), and also with milk fat (Ryan and Petit, 2010). Direct interactions between polyphenol and food

components such as proteins or polysaccharides can occur, and may affect the bioaccessibility and consequent absorption rate.

4. Conclusions

Rosmarinic acid is one of the major polyphenolic substances found in many culinary herbs. The potential health benefits of this compound have been well reported in both humans and animals studies, especially with regard to its antioxidant and anti-inflammatory capacities, as reviewed above. Consequently, there has been a growing research interest of inclusion of this polyphenol in the human diet, contributing to increase its nutritional and health benefits value.

Bioavailability and tissue distribution of RA are key factors that need to be clearly established in association with its biological effects. There is a complex interplay between RA beneficial properties and its pharmacokinetics, including absorption, distribution, metabolism and excretion, which determine its bioactive target sites in the body.

The beneficial use of RA as an additive food is limited by intrinsic features that lead to low bioaccessibility, low bioavailability and formulation challenges; one of the most serious being the biochemical interactions between RA with biological components of a food matrix, which may lead to a decreased accessibility and consequently low bioavailability. In addition, several other factors can affect the efficacy of RA, such as the harsh environment of the gastrointestinal tract (where chemical degradation or bacteria decomposition will occur), intestinal and hepatic

metabolism, cellular uptake, intracellular metabolism, accumulation in tissues, and biliary and urinary excretion.

Future prospectives should focus on strategies for enhanced efficacy of RA, which will depend on improved accessibility and bioavailability. Novel delivery systems, such as nanoparticles, may be a promising strategy and are worthy of further investigation to enhance RA bioavailability, thus improving nutritional quality of food and consequently exerting beneficial effects to human health.

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Conflict of interest statement

The authors have declared no conflict of interest.

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Table 1. Examples of herbal medicinal plants from *Lamiaceae* family, old *Labitae* family from different countries of origin which constitute a great source of RA.

Source	Concentration range (mg/g)[a]	Reference
Hymenocrater calycinus	2-13 (I)	
***Lavandula* spp.**	1.7 (I)	
Mellisa officinalis	36 (I); 0.8 (P)	
***Mentha* spp.**	24-58 (I)	
Mentha piperita	2.8 (U)	
Mentha arvensis	1.3-3.6 (P)	
Mentha spicata	3 (C)	Exarchou et al., 2002;
Origanum vulgare	31 (I); 13 (G)	Gohari et al., 2011;
Rosmarinus officinalis	7 (I)	Tahira et al., 2011;
Salvia officinalis	39 (I)	Shekarchi et al., 2012
Salvia fruticosa	14 (G)	
***Salvia* spp.**	4-16 (I)	
***Satureja* spp.**	3-19 (I)	
Satureja hortensis	21 (G)	
***Thymus* spp.**	0-31 (I)	
***Zhumeria* spp.**	2 (I)	

a) Ethanol extracts quantified by HPLC; C(Canada); I(Iran); G(Greece); P(Pakistan); U (Unknown)

Table 2. Examples of therapeutic applications considering the biological activities described for rosmarinic acid.

Application	Model / dose / frequency / route	Key notes	Ref.
Liver diseases	▪Sprague-dawley rat (weight 160–200 g) ▪Subcutaneously injected with CCl_4 to induce liver fibrosis ▪Intra-gastric administration ▪RA dosage - 2.5, 5 and 10 mg/kg ▪During 4 wks	▪ *In vivo*: reduction of TGF-β1 and CTGF expression in fibrotic liver; increment of serum ALB/GLB, decrease of serum levels of HA, LN and PCIII, and content of HYP in fibrotic liver. ▪ Improvement of liver function on liver fibrosis model rats	Li et al. (2010)
Liver diseases	▪Male Balb-c mice (7-9 wks old) ▪Oral administration ▪RA dosage - 135 mg/kg; an equivalent dose of PE (200 mg/kg)	▪ Inhibition of production of peroxinitrite and SOD ▪ Reduction of expression of TNF-α mRNA and iNOS ▪ Reduction of the plasma asparatate aminotransferase levels	Osakabe et al., 2002
Lung disease	▪Male ICR mice (6 wks old) ▪Oral administration ▪RA dosage- 2 mg/body weight 3	▪ Inhibition of DEP-induced formation of nitrotyrosine ▪ Reduction of pro-inflammatory	Sanbongi et al., 2003

	days prior to DEP treatment	molecule expression and ROS	
Skin cancer	▪Male Swiss albino mice (4-6 wks) ▪Oral administration ▪RA dosage- 100 mg/kg BW before to exposure of carcinogen DMBA ▪25 wks (3 times/wk on alternate days)	▪ Prevention of skin tumor formation ▪ Restore of status of lipid peroxidation and antioxidant status in DMBA treated mice	Sharmila and Manoharan, 2012

Table 2. Examples of therapeutic applications considering the biological activities described for rosmarinic acid (continued).

Application	Model / dose / frequency / route	Key notes	Ref.
Oral cancer	▪ Syrian hamsters ▪ Oral administration ▪ RA dosage - 100 mg/kg body weight	▪ Stimulation of activities of detoxification enzymes ▪ Improvement of status of lipid peroxidation and antioxidants ▪ Downregulation of the expression of p53 and bcl-2	Anusuya and Manohara n, 2011
Cardiovascular system	▪ H9c2 rat cardiomyoblast cell line treated with ADR to induce cardiotoxicity ▪ RA dosage 20 µg/mL	▪ Protection of cardiac muscle cells from ADR-induced cell death ▪ Anti-apoptotic effect ▪ Inhibition of ROS generation and JNK and ERK activation ▪ Reversion of the downregulations of GSH, SOD and bcl-2 by ADR.	Kim et al., 2005

Metabolic syndrome	▪ Wistar rats (weight 150-180 g) fed 60% of fructose diet ▪ Oral administration ▪ RA dosage 10 mg/kg/dia for 45 d	▪ Increase of insulin sensitivity, decrease of BP and prevention of cardiac damage ▪ Increase of circulatory and cardiac antioxidant capacity ▪ Promotion of NO production, downregulating ET-1 production and ACE activity in FFR ▪ Decrease of lipid levels and oxidative damage	Karthik et al., 2011

ACE, angiotensin-converting enzyme; ALB/GLB, albumin/globulin; BP, blood pressure; CTGF, connective transforming growth factor; DEP, diesel exhaust particles; ERK, extracellular-signal-regulated kinase; ET-1, endothelin-1; GSH, glutathione; HA, hyaluronic acid; HYP, hydroxyl proline; ICR, imprinting control region; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinases; LN, laminin; NO, nitric oxide; PCIII, collagen types III; SOD, superoxide dismutase; TGF-β1, transforming growth factor-beta1.

Table 3. Pharmacokinetics data of RA in the rat after different routes of administration.

Route/ Dose	Sample	Levels	Ref
• **I.V** • **60mg/kg of S. miltiorrhiza depside salts**	▪Plasma	▪ AUC $_{(0\text{-}6h)}$ (mg.h/l): 6.6; $MRT_{(0-\infty)}$ (h):0.32 ▪ $T_{1/2}$ (h):0.12; Clearance (L/h/kg):1.02	Li et al., 2007
	▪Heart	▪ Cmax:1.08; $T_{1/2}$ (h):1.39 ▪ $AUC_{(0\text{-}6h)}$ (mg.h/l): 0.34	
	▪Liver	▪ Cmax (µg/g):0.29 ▪ $AUC_{(0\text{-}6h)}$ (mg.h/Kg): 0.04	
	▪Spleen	▪ Cmax (µg/g):0.01 ▪ $AUC_{(0\text{-}6h)}$ (mg.h/Kg):0.07	
	▪Lung	▪ Cmax (µg/g):3.00; $T_{1/2}$ (h):0.19 ▪ $AUC_{(0\text{-}6h)}$ (mg.h/Kg):1.68	
	▪Kidney	▪ Cmax (µg/g):43.65(2007); $T_{1/2}$ (h):0.36 ▪ $AUC_{(0\text{-}6h)}$ (mg.h/Kg): 10.84	

	▪Brain ▪ Cmax (µg/g):0.06 ▪ $AUC_{(0\text{-}6h)}$ (mg.h/Kg):10.01	
• **Gastric intubation** • **RA:100µmol/kg**	▪ Cmax (µmol/l):1.36 ▪ Tmax(min):10 ▪ $T_{1/2}$(min):56.9 ▪ $AUC_{(0\text{-}1.5\ h)}$ (µmol min/L):60.4	Konishi et al., 2005

Table 3. Pharmacokinetics data of RA in the rat after different routes of administration (continued).

Route/ Dose	Sample	Levels	Ref
• **I.V** • **SA: 80 mg/kg**	▪Plasma	▪ $AUC_{(0-\infty)}$ (µg/ml.min):425.57 ▪ $MRT_{(0-\infty)}$ (min):51.43 ▪ $T_{1/2}$ (min):56.45±0.67	Lai et al., 2011
• **Oral** • **SA: 800 mg/kg**	▪Plasma	▪ $AUC_{(0-\infty)}$ (µg/ml.min):185.15 ▪ $MRT_{(0-\infty)}$(min):104.19 ▪ $T_{1/2}$ (min): 63.68 ▪ Tmax (min):10±0.33 ▪ Cmax (µg/ml):1.86	
• **Oral** • **RA: 50mg/kg**	Plasma	▪ RA: Cmax: 4.63 µmol/l -0.5 h ▪ Methyl-RA: 5.03 µmol/l – 1h ▪ COA: 0.75 µmol/l- 8h	Baba et al., 2004)
	▪Urine	▪ Sum of RA, methyl-RA, CAA, FA and COA including conjugated forms excreted by the urine within 18 hours after intake of RA of 5.47 % of the total RA dose. About 83% of this exception in the period 8 to 18h after RA administration.	

		▪ Total recovery of RA in urine of 0.44%	
• **Oral** • **RA: 200 mg/kg**	▪ Urine	▪ Main metabolites in Urine: m-hydroxyohenylpropionic acid, sulphated forms of COA ▪ Total cumulative amount of 6 metabolites and RA excretion after 48h after RA administration of 31.8%. ▪ Recovery of intact RA in the urine of 0.077% of the amount ingested	Nakazawa and Ohsawa, 2000

AUC, area under the curve; MRT, mean residence time; $T_{1/2}$, elimination half-life; Cmax, maximum plasma concentration; Tmax, time taken to reach the maximum plasma concentration; COA, m-coumaric acid; CAA, caffeic acid; FA, ferulic acid; I.V., intravenous administration.

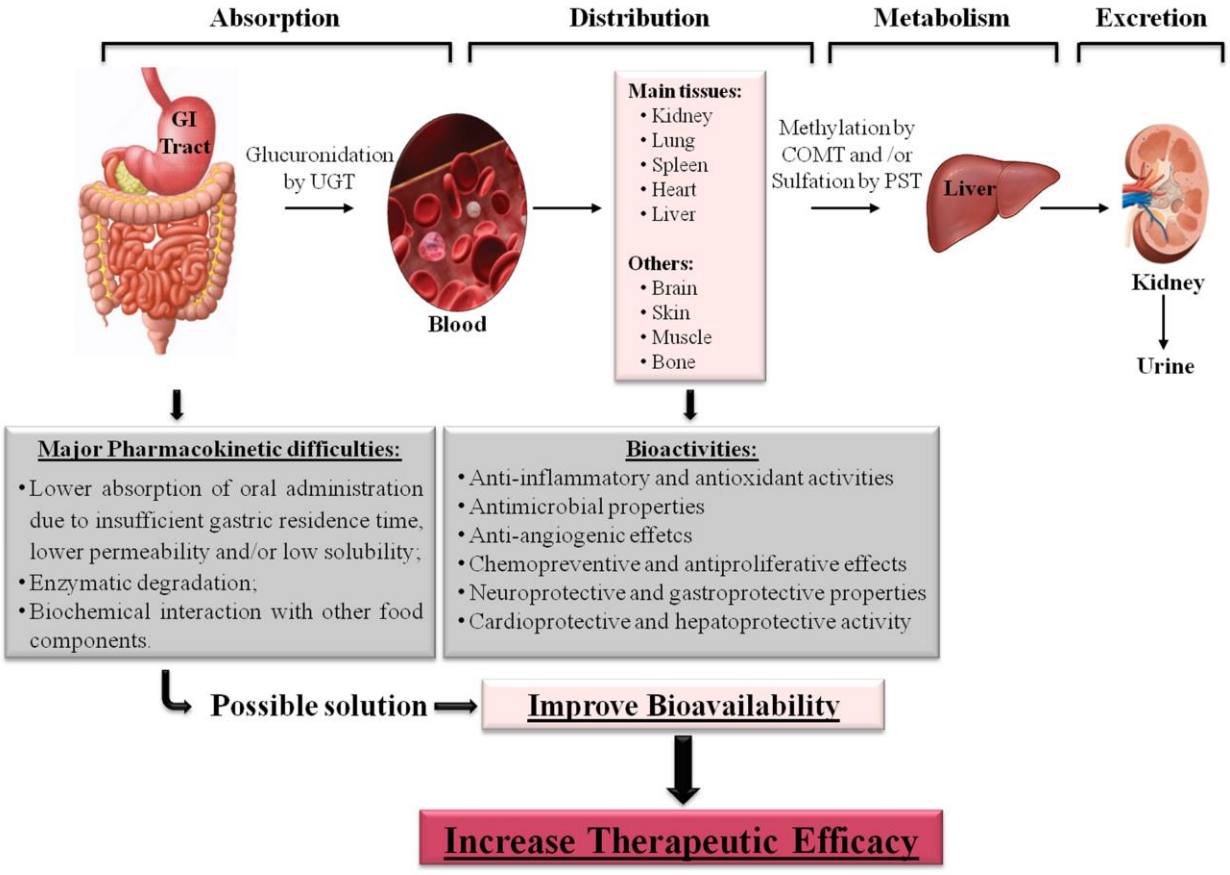

Fig.1. Phamacokinetic characteristics and main bioactivities of rosmarinic acid and major challenges to improve bioavailability and therapeutic efficacy. GI tract, gastrointestinal tract; UGT, UDP-glucuronosyltransferase; COMT, cathecol-*O*-methyltransferase; PST, phenolsulfotransferase