Introduction

Natural products are of a great importance due to their unique chemical diversity, what naturally results from diversity in their biological activities (Yuan et al., 2016). A lot of plant-originated drugs used in clinical medicine today were discovered through their previous application in traditional medicine (Fabricant & Farnsworth, 2001; Li-Weber, 2009). The secondary plant metabolites were derived through biodiversity phenomenon in which the interactions among organisms and their environment put together the diverse complex of chemical entities within the plants which further enhance their survival and competitiveness (Lee, 2010).

The great advances in molecular, biochemical and analytical methods allow separation of individual plant phytochemicals and consequently analysis of their chemical structure. In this context, natural products (NPs) are structurally diverse and serve as a val-
Flavonoids are a class of polyphenol secondary metabolites and till now more than 9000 flavonoids have been described (Hasten, 2002; Wang et al., 2015). They can be found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, tea and red wine. The term flavonoid is a collective noun for the plant pigments, mostly derived from benzo-γ-pyrone, which is synonymous with chromone. Different substrates are often bound on chromone what influence structure activity relationship (Middleton et al., 2000). Flavonoids have low molecular weight and are frequently found in glycosylated or esterified forms, consisting of C$_6$-C$_3$-C$_6$ rings (Isoda et al., 2014). Flavonoids attract considerable attention as valuable therapeutic option against a number of diseases and are subdivided according to their substrates into flavones, flavonols, anthocyanidins, flavanols, flavanones, flavononols, auroins, furan chromones, isoalloxazines, isoflavonones, biflavones, xanthones, chalcones and dihydrochalcones. Isoflavones represent by far the largest flavonoid subclass (Reynaud et al., 2005; Naguleswaran et al., 2006). This review focuses on four chemically different flavonoid compounds and summarizes present knowledge about their activities on cestodes and trematodes conducted in vitro and/or in vivo studies.

Selected flavonoids and their effects on flatworms

Curcumin
Curcumin ((1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5-Dione) is lipophilic polyphenol and is the major constituent in rhizome of Curcuma longa, a member of ginger family Zingiberaceae (Hatcher et al., 2008) (Fig. 1).

Following the administration, its decreased bioavailability supports its short half-life and extremely low serum and tissue concentrations. Curcumin undergoes extensive metabolism in liver and intestine (Shéhzad et al., 2017). According to the several studies, curcumin is well tolerated at doses up to 8 g/day/kg administered for a short period. However, the other studies showed that doses ranging from 0.9 – 3.6 g/day given from 1 to 4 months can/ may have undesirable effects such as nausea, diarrhea and may increase the level of lactate dehydrogenase and serum alkaline phosphatase. Other side effects in patients have also been recorded after a long-term exposure to higher doses of curcumin. Those include chest tightness, gastrointestinal upset, skin rash and infamed skin (Sharma et al., 2004). Curcumin is well known for a wide spectrum of its biological activities. Many clinical studies have confirmed anti-inflammatory effects of curcumin in onset of many diseases (see review of Kahkhaie et al., 2019). Shéhzad et al. (2017) concluded that curcumin binds to different molecular targets and affects various cell-signaling pathways in different diseases, including cancers, diabetes, cerebral edema, scleroderma, allergy and bronchial asthma, rheumatoid arthritis, neurodegenerative diseases, renal ischemia, cardiovascular diseases, psoriasis, obesity, and inflammatory bowel disease. Even though different curcumin biological activities are proved, its poor bioavailability...
due to poor absorption, rapid metabolism and systematic elimination represents a challenge for the optimization of its therapeutic efficacy such as, the utilization of various drug carriers or more soluble derivates (Anand et al., 2007).

The effects of curcumin on flatworms

Curcumin’s activity on cestodes

Raillietina spp. belongs to the parasitic tapeworms that infect the small intestine of chickens and occasionally other birds such as turkey and guinea fowl. El-Bahy and Bazh (2015) evaluated in vitro and in vivo anthelmintic activity of commercially available ginger root extract containing 5 % gingerols (Now, USA) and curcumin extract, containing 95 % curcuminoids (Earthstream Herbs, USA) against adults’ stage of cestode Raillietina (R.) cesticillus. Both extracts reduced the physical activity (movement) of R. cesticillus in concentration-time-dependent manner and eradicating of 65 – 80 % of worms was observed after 48 h exposures to curcumin at the concentrations of 25 mg/ml or 100 mg/ml, respectively. Antiparasitic activity of extracts in the infected chickens was lower after administration of 1000 mg of curcumin or 500 mg of ginger where 40 – 50 % of worm’s survival was observed. Deleterious effect of both extracts was firstly manifested on worm’s tegument and authors thought that after the extract absorption/penetration effect of both extracts was firstly manifested on worm’s tegument where 40 – 50 % of worm’s survival was observed. Deleterious effect of both extracts was firstly manifested on worm’s tegument and authors thought that after the extract absorption/penetration interfered with the glucose metabolism in the worms, thus leading finally to their killing. In general, glucose and other simple carbohydrates are the main energy source for the cestodes and trematodes (Roberts, 1983). Lower efficacy in vivo may be due to the differences between pH in culture medium and pH in the animal’s stomach/intestine, what can influence the absorption and pharmacokinetics of these lipophilic compounds (El-Bahy & Bazh, 2015).

Curcumin’s activity on trematodes

Fasciolosis is an economically important global disease of ruminants in the temperate and tropical regions. It is caused by Fasciola (F.) hepatica and F. gigantica, respectively, and also presents a potential zoonotic threat. Ullah et al. (2017) examined the anthelmintic potential of thymoquinone and curcumin on adult flukes of F. gigantica in vitro. A significant reduction in the worm motility and severe disruption of fluke tegumental surface was observed at the 60 μM concentration for both compounds. Curcumin was more potent in the reduction of activity of fluke’s antioxidant enzymes glutathione-S-transferase and superoxide dismutase as well as in reduction of glutathione (GSH) levels. Results showed that both compounds caused primarily alterations in tegument and tegumental disruption what can also affect the energy dependent Na+ – K+ transport leading probably to the swelling of worm’s syncytium, subsequently reducing parasites motility. The tegumental damage along with trans-tegumental uptake affects excretory/secretory processes, changes the signaling pathways and also impacts the metabolic pathways (Halton, 2004).

Higher plasma levels of insoluble compounds can be achieved after their entrapment in a suitable carrier. Luz et al. (2012) prepared poly(lactic-co-glycolic acid) (PLGA) nanoparticles with incorporated curcumin and demonstrated that 100 μM of curcumin drug formulation caused surface alterations followed by death of all Schistosoma (S.) mansoni adult worms in vitro. In addition, PLGA-curcumin particles reduced also the worm’s motor activity. The effects of polylactic acid (PLA) nanoparticles loaded with curcumin-nisin were examined on oviducal activity and reproductive capacity of Fasciola spp. in vitro. It was shown that PLA nanoparticles with curcumin at the concentration of 5 mg/ml lead to decrease of percentage in egg hatching to a 41.7 % when compared with the positive control group treated with albendazol only (45.1 %). Aberrations observed in sperm cells were not significantly different between examined groups (Oyeyemi et al., 2018).

The potential in in vitro schistosomicidal effects of pure curcumin was for the first time confirmed by Magalhães et al. (2009) on S. mansoni. After the exposure to 50 μM and 100 μM of the compound, all worms were found dead. Meanwhile lower doses (5 μM and 20 μM) decreased worm viability in comparison with the positive and negative control groups. Moreover, all pairs of coupled adult worms were separated into individual male and female by curcumin at the doses of 20 μM to 100 μM. Significant reduction in egg production by 50 % in comparison with positive control group was found after exposure to 5 μM and 10 μM concentrations. Mo-rais et al. (2013) in their in vitro study on S. mansoni demonstrated that curcumin modulates activity in many genes. More than 2374 genes were significantly and differentially expressed. Those counted were involved in regulating of important signaling pathways which affect embryogenesis and oogenesis, such as Notch and TGF-β (transforming growth factor β) (Sethi & Kang, 2011; Moskowitz & Rothman, 1996).

Abou El Dehab et al. (2019) monitored in vitro effects of curcumin on adult S. mansoni and S. haematobium viability, the tegument ultrastructure and egg hatchability. High doses of curcumin (500 μM) resulted in 100 % irreversible killing of both Schistosoma species after 2 h of incubation and at 50 μM concentration, the all pairs of worms were separated into individual male and female. Curcumin had stronger schistosomicidal effects on S. haematobium than on S. mansoni, and at concentrations 125 – 500 μM, it disrupted the shell wall of parasite’s eggs, thus allowing the untimely escape of the miracidium and leading to its consequent death. Tegumental alterations caused by curcumin exposure probably modulated calcium level in worms by stimulation of Ca2+ uptake and reduction of Ca2+ leakage. Consequently, inhibition of IP3 (inositol triphosphate)-induced Ca2+ release from endoplasmic reticulum affected Ca2+-dependent cellular events (Dyer et al., 2002; Zhang et al., 2014). Moreover, eggs hatchability and viability were also significantly affected by curcumin consequently supporting its potential for use in the therapy of S. mansoni and S. japonicum infections taking the advantage of its increased bioavailability in the gastrointestinal tract.

The ROS mediated apoptosis seems to be an effective strategy
to control parasitic infections including the helminth parasites. The \textit{in vitro} treatment of \textit{F. gigantica} worms with curcumin at 60 μM, resulted in increased generation of reactive oxygen species (ROS) whereas the level of reduced glutathione, a primary redox regulator, was found to be significantly decreased (p < 0.05) (Rehman et al., 2020). It also inhibited the sigma GST at transcriptional and translational level, which is an important detoxification enzyme and also a key drug/vaccine target. Moreover, curcumin significantly inhibited the activity of antioxidant enzymes glutathione peroxidase and glutathione reductase that are vital in maintenance of redox homeostasis. The oxidative stress along with induction of apoptotic-like events would compromise the survival ability of worms within the host. It was found that two essential antioxidant enzymatic systems thioredoxin and glutathione, which occur in all organisms, differ in parasitic and free-living Platyhelminthes (Otero et al., 2010). Authors found that parasitic Platyhelminthes possess a unique and simplified redox system called thioredoxin-glutathione reductase (TGR) for diverse essential processes, which is excellent drug target.

The induction of apoptotic death in parasites by drugs is another important parameter for the reduction of infections. The oxidative stress is harmful to worms as it causes modification in cellular macromolecules and could alter the normal function of key enzymes / proteins, and also promotes cell death. Curcumin was shown to generate oxidative stress, induce apoptosis, DNA damage and fragmentation in adult \textit{S. mansoni} worms \textit{in vitro}. DNA fragmentation appears to be the sign of undergoing cell apoptosis. Authors confirmed the increment in expression of SmCASP3/7 transcripts and the activity of caspase 3. Though, the activity of caspase 8 was not affected after curcumin treatment (De Paula Agular et al., 2016). The \textit{in vivo} schistosomicidal activity of this polyphenol was also confirmed experimentally in mice infected with 80 \textit{S. mansoni} cercariae which were injected intraperitoneally with curcumin at a total dose of 400 mg/kg body weight (Allam, 2009). Curcumin was effective and responsible for significant worm reduction and tissue-egg burdens, hepatic granuloma volume, liver collagen content, and restored hepatic enzymes activities to the normal levels, and enhanced catalase activity in the liver tissue of infected mice. Moreover, treatment modulated the IL-12 and TNF-α cytokine levels and augmented the production of IgG1 antibodies specific to worm antigens.

Present reports highlight the potential of curcumin to be used as addition to the primary therapy of cestode and trematode infections utilizing its direct wormicidal activity in conjunction with its health-promoting activity (Fig. 2).
Genistein

Genistein (5, 7-dihydroxy-3-(4-hydroxyphenyl) chromen-4-one) is an isoflavonoid compound, naturally occurring in plants of the soy family, belonging to a group of nutraceuticals (Tandon & Das, 2018) (Fig. 3). However, after the fermentation and digestion it is metabolized from isoflavone glycoside to isoflavone aglycone (Markovits et al., 1989).

Fig. 3. Structure of genistein. (https://pubchem.ncbi.nlm.nih.gov/compound/5260961#section=2D-Structure)

Genistein was for the first time isolated from Genista tinctoria, Fabaceae in 1899 and is present in high concentration in soybean – a high protein legume. Genistein has a similar structure as native estrogens, can act like estrogen-agonist (Brzezinski – a high protein legume. Genistein has a similar structure as native estrogens, can act like estrogen-agonist (Brzezinski, 2008) (Fig. 3). However, after the fermentation and digestion it is metabolized from isoflavone glycoside to isoflavone aglycone (Markovits et al., 1989).

Genistein's activity on cestodes

In general, cestodes and trematodes treatment in vitro with genistein causes worm’s immobilization and flaccid paralysis in dose-dependent manner suggesting that genistein acted primarily on the flatworm’s tegument, where it interacts with enzymes and induces irreversible structural alterations (Tandon et al., 1997). The array of its activities on flatworm’s physiology indicates that it can pass through tegument.

The effects of genistein and a number of synthetic genistein derivatives on metacestode stage of cestodes Echinococcus multilocularis and Echinococcus granulosus were investigated in the study of Naguleswaran et al. (2006). In vitro treatment with genistein at the concentrations of 5 and 10 μg/ml for 7 days led to the profound morphological and structural alterations in both Echinococcus species. Moreover, authors showed that two synthetic genistein derivatives carrying a modified estrogen receptor binding site were also able to induce dramatic breakdown in the structural integrity of the metacestode germinal layer at the concentration (1 – 10 μg/ml) as it was indicated by concentration dependent release of tegumental enzyme-alkaline phosphatase in vitro. This resulted in decreased viability and subsequent death of parasites. Authors suggest that inhibition of protein kinases which regulate downstream signaling pathway involving mitogen-activated protein kinase is accounted, at least in part, for cestocidal effects of these compounds.

The multifunctional effect of genistein on flatworm physiology was demonstrated in several in vitro studies utilizing bird’s cestode model of R. echinobothrida. Ca2+ takes part in a muscle contraction during cestodes adult and metacestode stages (Bryant & Behm, 1989) and impacts regulation of several enzymes such as glycogen phosphorylase, glycogen synthase or protein kinase (Bollen et al., 1998; Nelson & Cox, 2000). Das et al. (2006) studied the effect of genistein and root-peel extract of F. vestita on Ca2+ homeostasis in R. echinobothrida in which the significant amount of Ca2+ and several other metal ions was found. The cestodes were incubated with root-peel extract (5 mg/ml), genistein (0.2 mg/ml) or praziquantel (0.001 mg/ml) for 6 h and treatments led to decrease in Ca2+ concentration by 49 %, 39 % and 45 %, respectively. In comparison with the parallel control group an increase in Ca2+ efflux by 100 %, 118 % and 94 % was observed. The results suggest that the changes in the Ca2+ homeostasis can induce rapid muscular contractions leading to the parasite paralysis and may result in anthelmintic stress caused by herbal components. Muscle activity is regulated also by NO (nitric oxide) which is synthesized in the nervous system by nNOS enzyme. NO is a unique neuronal messenger and among other properties it possesses an anthelmintic
**Ca²⁺ homeostasis**

- Flaccid paralysis (Lynden et al., 2008; Toner et al., 2008)
- Immobilization (Yadav et al., 1992)
- Morphological and structural alterations in tegument (Tandeon et al., 1997; Nageluswaran et al., 2006)

**Ca²⁺ efflux**

- In the medium, muscular contractions, parasite paralysis (Das et al., 2006)

**NO**

- NADPH-d, histochemical marker for nNOS (Kar et al., 2002)
- Activity of nNOS (Das et al., 2007)
- NO efflux
- cGMP, mediator for NO (Das et al., 2007)

**carbohydrates metabolism**

- Changes of glycogen metabolism (Tandon et al., 2003)
- Changes of HMP and gluconeogenesis (Das et al., 2004)
- Changes in carbohydrate metabolism in cestodes (Tandon and Das, 2007)
- Changes in carbohydrate metabolism in trematodes (El-Ansary, 2007)

**fibrotic diseases**

- Collagen in trematodes (Sophy et al., 2018)
- Expression of TGF-β1 (Sophy et al., 2018)

---

**Genistein’s activity on trematodes**

The effect of genistein on the nNOS and NO concentrations was demonstrated also in trematodes *in vitro*. The NADPH-diaphorase (NADPH-d) is a histochemical marker for the nNOS and *Kar* demonstrated also in trematodes *in vitro*. The NADPH-diaphorase reaction in the fluke *Fasciolopsis buski* treated with the root peel extract of *F. vestita* and genistein. This histochemical marker was evident in neuronal cell bodies, cerebral ganglia, the brain commissure, main nerve cords, and in the innervation of the pharynx, ventral sucker, terminal genitalia as well as genital parenchyma of the worms. Genistein treatment *in vitro* resulted in alterations of free amino acid pool and ammonia levels in the same trematode species (Kar et al., 2004).

Reviewing of literature revealed that information is missing about the direct *in vitro* effects of genistein on *Schistosoma* spp. The few *in vivo* studies on *S. mansoni* and *S. japonicum* on mouse-models demonstrated its beneficial effect in the therapy as the result of its effect (Mahmoud & Habib, 2003). NO generated from amino acid L-arginine and molecular oxygen by NOS enzymes (nitric oxide synthases) can be divided into neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) (Moncada et al., 1991). It was shown that genistein has a potentiating effect on the nNOS activity, NO efflux and the cGMP concentration (cyclic guanosine monophosphate), which works as a mediator for NO (Das et al., 2007). The activity of nNOS was significantly increased by 36 – 46 % and NO efflux was augmented 2-fold in the incubation medium in a group of worms exposed to genistein (0.2 mg/ml) for various time-periods when compared to the control group. Changes in nNOS activity correlated with the increase of cGMP concentration by 46 – 84 %.

Carbohydrates, stored in the form of glycogen, are the major energy source for cestodes and trematodes (Smyth et al., 1991). It was shown that genistein influences metabolism of glucose and glycogen was further investigated in the study of Tandon and Das (2007) on the same cestode model *in vitro*. Worms exposed to genistein at a concentration of 0.2 mg/ml were able to renew the missing energy from glycogen reserves by GPase activation (glycogen phosphorylase) and GSase (glycogen synthetase) activity inhibition, where deficiency of glucose led to the activation of PEPCK (phosphoenolpyruvate carboxykinase) – malate pathway.

To date, the information are lacking regarding *in vivo* antiparasitic effect of genistein on the cestode infection and its activity on pathophysiology of the hosts. The anthelmintic effects of genistein in flatworms are summarized in Fig. 4.

---

**Fig. 4. Anthelmintic effects of genistein on flatworm’s tegument, NO, Ca²⁺ homeostasis, carbohydrates metabolism and fibrotic diseases.**
direct effect on worms and possibly by modulation of pathological outcomes of infection. In schistosomiasis, the egg deposition in the liver contributes to the formation of hepatic granuloma and fibrosis, which are the most serious clinical pathological features. Sobhy et al. (2018) examined antischistosomal and antifibrotic activity of genistein in acute and chronic experimental S. mansoni infection in comparison to praziquantel treatment. The reduction in the percentage of collagen in both acute and chronic stages and also the reduction in the expression of TGF-β1 in the examined hepatocytes in the both stages were observed. According to the results, genistein, mainly in combination with praziquantel, may protect from S. mansoni-induced liver damage, and reduce the development of fibrosis. It has been proposed that activation of the nuclear factor kappa B (NF-κB) signaling pathways is closely associated with the development of hepatic granuloma and fibrosis. Genistein has been shown to inhibit the activity of NF-κB signaling pathways in many cells. In BALB/c mice infected with S. japonicum the activity of NF-κB signaling and inflammatory markers MCP1 and TNF-α declined sharply after the treatment with genistein what correlated with reduced S. japonicum egg-induced liver granuloma and fibrosis (Wang et al., 2018). This implies that genistein can be a potential natural agent against schistosomiasis.

Quercetin
Quercetin (3, 3’, 4’, 5, 7-pentahydroxyflavone) is natural compound of flavonoid type (Fig. 5) occurring in low amounts in fruits (e.g. cranberries, cherries, grapes) and vegetables (e.g. onion, peppers, asparagus). Its antioxidant, anti-inflammatory, immunoprotective or anticarcinogenic effects are well described (Andres et al., 2018).

After the oral administration, quercetin glycosides, mainly quercetin aglycon, may passively permeate through intestinal epithelial barrier and they could also be transported by intestinal sodium/glucose cotransporter-1 (Murota & Terao, 2015; Andres et al., 2018). Quercetin is extensively metabolized in the enterocytes and further in the liver forming a plethora of metabolites (Graefe et al. 1999; Wang et al., 2016). Therefore, it is highly probable that described health-promoting and antiparasitic effects are results of synergistic action of various metabolites. This, however, possesses disadvantages in the evaluation the mechanisms by which quercetin or its metabolites interfere with molecular targets in flatworms. Moreover, the pharmacokinetics can show a high inter-individual variability, depending on, for example, genetic variation, individual antioxidant status and the other factors (Guo & Bruno, 2015).

Quercetin’s activity on trematodes
In the natural medicine the plants Styrax camporum Pohl and S. pohlii A. DC. (Styracaceae) are used for the treatment of gastrointestinal diseases and fevers, respectively. Braguine et al. (2012) reported for the first time the presence of quercetin and also the other flavonoid kaempferol in ethyl acetate fraction of aerial parts of Styrax camporum. Evaluation of the schistosomicidal activity on S. mansoni adult flukes in vitro revealed that worms incubated with 100 μM of quercetine exhibited moderately reduced parasites motor activity, without tegumental alterations. On the other hand, kaempferol did not show any tegumental alterations and motor activity but was able to completely separate adult worms into males and females. The mechanism by which flavonol derivatives, exert their in vitro schistosomicidal effect is not clear. However, quercetin was identified as a selective inhibitor of the S. mansoni NAD+ catabolizing enzyme (SmNACE), which is localized on the outer surface ( tegument) of the adult parasite (Kuhn et al., 2010).

Momordica charantia is considered as important medicinal plant by some African and Asian communities. The presence of quercetin as the major constituent was confirmed in crude extract of this plant by HPLC analysis and subsequent mass-spectrometric analysis (Pereira et al., 2016). Authors examined the effect of the crude extract and sub-fractions on the embryonic development of F. hepatica eggs in vitro. After 12 days no larvae were formed from the eggs incubated with extract at the concentrations above 12.5 mg/ml. Sub-fractions at concentrations between 0.01 and 1000 μg/ml affected differently trematode’s embryonic development, and n-butanol fraction containing the highest proportion of quercetin induced the strongest inhibition of miracidia formation. Authors concluded, that the presence of another compounds beside quercetin in the crude extract of M. charantia are also important, where the synergic effect of all components may lead to the final antiparasitic effect of this extract.

To date, no reports on the activity of quercetin on cestodes or cestodiasis have been published. Anthelmintic effects of quercetin are summarized in Fig. 6.

Silymarin’s complex of flavonoids
Silybum marianum (Asteraceae) known also as milk thistle is a plant of the Asteraceae family and its seeds are the source of sily-
marin - a unique complex of flavonolignans and other polyphenols. The main representatives of this group presented in silymarin are silybin, isosilybin, silychristin, isosylichristin and silydianin (Fig. 7). In addition to the above mentioned flavonolignans, silymarin contains also other constituents (e.g. taxifolin, dihydrosilybin, dihydrokaempherol, kaempherol, naringin, eriodyctol, chrysoeriol), and many other molecules in a very low concentrations (Abenavoli et al., 2018; Anthony & Saleh, 2013). The major bioactive compound in silymarin extract is silybin, which forms up to 40 % of the content and depends on many factors (Lee et al., 2007; Chambers et al., 2017). Silybin is a mixture of two diastereoisomers: A and B what can be separated by high-performance liquid chromatography (HPLC). Due to low water solubility (20 – 50 %) silymarin is after oral administration absorbed from gastro-intestinal tract where undergoes extensive enterohepatic circulation (Saller et al., 2008; Javed et al., 2011). Pharmacokinetic study in rats showed that orally administered silybin (50 mg/kg) had good tissue distribution profile reaching micro molar concentrations, e.g. 8.8 μg/g

Fig. 6. Anthelmintic effects of quercetin.

Fig. 7. Structures of selected representatives of silymarin complex.
of livers (Zhao & Agarwal, 1999). The milk thistle is a medicinal plant used for more than 2000 years to treat a wide range of liver and gallbladder disorders, including hepatitis, cirrhosis and jaundice. Protect the liver from poisoning with chemical and environmental toxins. Due to these effects, silymarin was by the WHO in the 1970s classified as an official medicine with hepatoprotective properties (Wesolowska et al., 2007).

Many scientific teams are interested in multiple effects of silymarin/silybin of which probably the best characterized is its antioxidant effects. Surai (2015) reviewed various routes and mechanisms of silymarin antioxidant actions in animal models and in human trials. It has been demonstrated that there are strong direct scavengers of some free radicals (Dehmlow et al., 1996). It is well documented, that silymarin/silybin can decrease oxidative stress and has a protective effect on mitochondrial structure (Rolo et al., 2003) and immune cells function (Hrčková et al., 2020a). There are also well documented antioxidant protective properties of silymarin/silybin in the prevention of toxic effects of various chemicals, such as arsenic, carbene tetrachloride, mycotoxins, thioacetamide, cisplatin, manganese, etc. (Surai, 2015). The mechanisms of silymarin hepatoprotective activities are well recognized. For example, Heidarian and Nouri (2019) confirmed the protective effects of silymarin on diclofenac-induced liver toxicity and oxidative stress in male rats. A growing number of studies have shown that silymarin/silybin exhibit also anticarcinogenic, immunomodulatory and anti-angiogenic activities (Gažák et al., 2007; Esmaeil et al., 2017).

**Silymarin/silybin activity on trematodes**

To date, the direct in vitro activity of silymarin or individual silymarin’s flavonolignans on trematodes has not been documented. However, there is an increasing number of studies reporting on the effect of silymarin administration alone or in combination with praziquantel on the infections induced by Schistosoma species in vivo (for example: El-Lakkany et al., 2012; El-Sayed et al., 2016; El-Hawary et al., 2018).

Up to now there have been several reports focused on the interactions between silymarin and individual compartments of immune system during schistosomiasis. In particular, a number of immunomodulatory activities on the infected hosts resulting in amelioration of the parasite induced pathology were reported. Kamel (2016) studied the anti-inflammatory and antifibrotic effects of silymarin alone or when combined with mefloquine during acute mouse model of schistosomiasis. It was confirmed that combined treatment can significantly reduce granulomatous reactions and hepatic fibrosis. Antifibrotic effect of silymarin administration in the livers was documented also in other studies. Silymarin caused a significant reduction in granuloma areas in S. mansoni infected mice when compared to controls (Tousson et al., 2013; Mata-Santos et al., 2010). Initiation of fibrosis and its perpetuation is an immunologically regulated process involving many cell types, cytokines and other mediators. Tousson et al. (2013) studied the histopathological and immuno-histochemical expression of apoptotic proteins P53 and CD68 marker present on myeloid lineage in the mice livers infected with S. mansoni and examined the protective role of silymarin. A profound increase in P53 and CD68 was found in the liver tissue after the infection in comparison to the control. In contrary a significant decrease in the expression of both pro-apoptotic proteins was observed after silymarin treatment. Cytokines are produced by various cell types and are important fibrosis regulators. The TGF-β1 is the key growth factor involved in fibrosis progression, and is responsible for differentiation of fibroblast to myofibroblasts. The IL-4 induces collagen synthesis and together with IL-13 can drive the differentiation of resident fibroblast and recruited fibrocytes to myofibroblast in a wide range of tissues (Mattey et al., 1997). IFN-γ inhibits fibrosis by antagonizing the pro-fibrotic activity of TGF-β1 whereas the TNF-α is proinflammatory cytokine which activation must be tightly controlled because it can lead to the host-tissue damage (Szekanecz & Koch, 2007). The levels of IL-4, TNF-α, TGF-β1 cytokines were significantly increased in S. mansoni infected group and treatment with silymarin alone or combined with praziquantel resulted in a significant decrease in IL-4, TNF-α and TGF-β1 levels and subsequent significant elevation in serum IFN-γ levels (El-Sayed et al., 2016). Based on the experimental results the treatment with silymarin combined with praziquantel could reduce hepatic fibrosis also by downregulation of profibrotic cytokines due to its immunomodulatory activity on various cell types in murine schistosomiasis. In the study of Mata-Santos et al. (2010) the parasite oviposition capacity was not affected by silymarin treatment. However granulomatous peri-ovular reaction and fibrosis in the liver had been reduced. Results showed that treatment with silymarin in acute phase of schistosomiasis could lead to a mild course of murine schistosomiasis. In the next study (Mata-Santos et al., 2014) authors focused on the changes of profibrogenic cytokines levels in liver and hepatic fibrosis during chronic murine schistosomiasis. Correspondingly, silymarin treatment reduced liver weight, granuloma sizes and fibrosis, what correlated with lower serum levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) in the liver: Alongside with reduction of IL-13 cytokine levels and increased levels of IFN-γ. The strong down-regulation of fibrosis in mice livers infected with S. mansoni after silymarin and praziquantel administration was confirmed also by El-Lakkany et al. (2012). In livers the partial decrease in worm burden, hepatic tissue egg load associated with an increase in percentage of dead eggs, modulation in granuloma size were documented. Moreover, a significant reduction in hepatic hydroxyproline content, the marker of fibrosis, was observed. The alleviated pathology was manifested by decreased expression of MMP-2 (matrix metalloproteinase-2), TGF-β1 and the number of mast cells. The elevation of reduced glutathione (GSH) levels was also detected.

Taken together, all these results suggest that treatment with silymarin in combination with praziquantel could be a safe and more effective treatment possibility for schistosomiasis what resulted in
amelioration of liver fibrosis and more effective wormicidal effect. Studies also showed that silymarin therapy has many beneficial immunological effects, what further contributed to the suppression of parasitic infection. Even though the direct parasitocidal effect of individual silymarin’s flavonolignans is also possible, and warrant further examinations.

**Activity of silymarin’s flavonolignans on cestodes**
Tetrathyridia of cestode *Mesocestoides (M.) vogae* represent metacestode stage and are considered as suitable model for the evaluation of larvicidal potential of various compounds. The possibility of axenic cultivation of parasites is a special feature for this model and was used to assess the *in vitro* effects of three silymarin’s flavonolignans – silybin, 2,3-dehydrosilybin and silychristin given at concentrations of 5 and 50 μM under aerobic and hypoxic conditions for 72 h (Hrčková et al., 2018). Under both sets of conditions, the silybin and silychristin suppressed the metabolic activity, concentration of glucose, lipids and partially motility, but the other hand neutral red uptake was elevated. The dehydrosilybin exerted larvicidal activity and affected the motility and neutral lipid concentrations depending on the cultivation conditions, whereas it decreased glucose concentration. Dehydrosilybin at the 50 μM concentration caused irreversible morphological alterations along with damage to the metacestodes microvillus surface. Authors concluded that silybin and silychristin suppressed mitochondrial functions and energy stores, thus causing a physiological misbalance. Dehydrosilybin exhibited a direct larvicidal effect due to the tegument damage and triggered complete disruption of larval physiology and metabolism.

The administration of silymarin (30 mg/kg/day) for 10 days as an adjuvant to the primary therapy with praziquantel given at the same doses enhanced the anthelmintic effect of drug in mice infected with the same metacestodes – *M. vogae* tetrathyridia (Veřebny et al., 2008, 2010). Reduced parasite burden in mice livers and peritoneal cavities was significantly higher after combined therapy, and correlated with profoundly decreased ALT (alanine aminotransferase) and AST (aspartate aminotransferase) activities in the serum. Similar association was observed with the hyaluronic acid level, albumin and total protein concentration. Authors showed that silymarin caused higher fibrosis suppression in the liver after combined therapy along with reduction of oxidative stress and inflammation. This was demonstrated by a lesser lipid peroxidation and elevation in GSH content and decreased hydroxyproline content. Similar readouts were observed in mouse nematode infections, where specific silymarin immunomodulatory effect was examined in extended study performed on mice infected with *M. vogae* (Hrčková et al., 2020b). Co-administration of silymarin modified the effects of praziquantel therapy. The antigenic stimulation of the immune system modulated the levels of Th1/Th2/Tregs cytokines in the serum, and altered gene expression in the livers, what was accompanied with reduced fibrosis.

In summary, silymarin and its main constituent silybin, can be considered as a very effective non-toxic compound for the therapy of flatworm infections where they noticeably potentiate the anthelmintic effect of drug via multiple mechanisms. The reduction of fibrosis other than the direct wormicidal effect, seen already at low concentrations, is also likely.

**Discussion**

Microbial metabolites and biologically active substances from plants are gaining increasing attention as potential parasitocides. Particularly, after a very successful introduction of commercial treatment with avermectins and milbemycins (Shoop et al., 1995). Discovery of new compounds effective against flatworm infections in both humans and animals, belonging to classes Cestoda and Trematoda is an urgent research goal. At present, due to the difficulties and research costs for discovery of new chemical entities a limited range of anthelmintics is available for the treatment. Basically only benzimidazole carbamates and praziquantel are widely used. Several research groups therefore focus on the evaluation of secondary plant metabolites referring to ethnopharmacological experiences using several species of cestodes and trematodes. In this review, we have summarized information about four flavonoids (curcumin, genistein, quercetin and silymarin complex) gained within the period of past three decades. Numerous studies on various natural products including flavonoids revealed that, in general, they can interact with multiple targets in eukaryotic cells, mostly proteins (enzymes, receptors, etc.) in dose-dependent manner. They usually have health beneficial effects against many diseases and interestingly, some of them also possess cestocidal or trematocidal activities *in vitro* and *in vivo*. Usually, a significant parasitocidal activity *in vitro* is achieved in micromolar concentration within the range from 5 up to 500 μM. Though, low bioavailability of water insoluble flavonoids can prevent to achieve its higher concentrations in the tissues of hosts. A group of abundant secondary metabolites in many plant species with reported bioactivity for virtually any biological/pharmaceutical endpoint have received the term “invalid metabolic panaceas – IMPS “ or “pan assay interference compounds – PAINS”, and include also the compounds addressed in present review. The term PAINS is used for compounds which typically interact nonspecifically with proteins in a high percentage of bioassays (Bisson et al., 2016; Courtney, 2017). But, interestingly, over 60 FDA-approved and worldwide drugs contain PAINS chemotypes (Kilchmann et al., 2016). Their multi-target activity may represent a serious problem in the development of a very specific antiparasitic drug according to the standard protocols in drug-discovery research. However, the development of novel chemical structure with high activity against metazoan parasites is a complicated, very costly task and also challenge for both the chemists and biologists. The very important issue is also toxicity of the potent drug to the hosts which is usually neglected in case of plant – derived compounds such as flavonoids (Hewitson et al., 2009).
In case of compounds where anthelmintic activity was demonstrated in vitro at relatively low concentration, the further research is often directed towards preparation of synthetic derivates with modified structure which could bind to a specific target on parasites. For example, synthetic derivate of genisten Rm6423 carries a modified estrogen receptor binding site but retains ability to target epidermal growth factor tyrosine-kinases in E. multilocularis and E. granulosus protoscoleces at lower concentration (Naguleswaran et al. 2006). The other example are 2, 3-dehydroderivates of flavonolignans silybin, silychristin and silydianin, which have antiradical and cytoprotective activity (Pyszkova et al., 2016). In our previous in vitro study, treatment of other metacone species M. vogae with silybin, silychristin and dehydrodiosilybin revealed that dehydrodiosilybin exhibited a direct larvicidal effect due to tegument damage and complete disruption of larval physiology and metabolism. Silybin and silychristin suppressed mitochondrial functions and energy stores, thus inducing a physiological misbalance (Hrčková et al., 2018).

A number of studies performed on several flatworm species with isoflavone genisten indicate that they can interfere with several parasite enzymatic systems, including enzymes involved in glucose metabolism (Tandon & Das, 2018). Similarly, silymarin flavonolignans at low concentration (5 μM) were able to decrease metabolic activity and glucose content in M. vogae tetrathyridia (Hrčková et al., 2018). This indicates that energy-generating enzymatic systems in mitochondrial respiratory chains in flatworms can be the targets for selected flavonoids. El-Bahy and Bazh (2015) examined effect of curcumin on adult stage of cestode Raillietina (R.) cesticillus in vitro. The authors thought that after absorption/penetration the extracts itself interfered with worm’s glucose metabolism and thus lead to their killing.

In general, helminths exploit a variety of energy-transducing systems during their adaptation to the peculiar habitats in their hosts, where differences in energy metabolisms between the host and helminths are attractive therapeutic targets. The majority of parasites, including flatworms, do not use the oxygen within the hosts, but employ systems other than oxidative phosphorylation for ATP synthesis (Sakai et al., 2012; Matsumoto et al., 2008). They often live in niche where oxygen tension is low therefore many of them exploit a unique anaerobic respiratory chain, called NADH-fumarate reductase system – complex II. It is a unique enzymatic system for energy generation, not found in normal eukaryotic cells, except for cancer cells (Tomitsuka et al., 2012). This respiratory chain was studied in detail in Ascaris suum nematode (reviewed in: Kita & Takamiya, 2002). The novel compound nafuredin isolated from Aspergillus niger mold inhibited complex II in this nematode in N concentrations in vitro (Sakai et al., 2012). Thus differences between parasite and host mitochondria hold great promise as targets for the therapy. However, so far activity of mentioned flavonoids or its derivatives on NADH-fumarate reductase system in flatworms has not been examined. The presence of this system was demonstrated in protoscoleces of Echinococcus species (Matsumoto et al., 2008). Interestingly, complex II is the main site of ROS production, which contributed to the pathology during infections, and was demonstrated in A. suum adult respiratory chain (Paranagama et al., 2010). Taking into consideration the anti-oxidant effects of flavonoids, their co-administration might alleviate pathology directly as scavengers of ROS. Their activities on this specific enzymatic system in flatworms await further research.

Apart of documented in vitro effects on flatworms summarized in this review, in vivo studies on model flatworm infections with Schistosoma species, Echinococcus species, M. vogae infection (and others) using flavonoids curcumin, genisten and mostly silymarin in combination with the anthelmintic drugs refer to their pleiotropic mode of action in the hosts. Moreover, besides the beneficial effects on the hosts, flavonoid co-administration contributed to the increased efficacy of the drugs. Reports cited in present review showed that silymarin contributed to the increased efficacy of praziquantel in S. mansoni and M. vogae infected mice. Collectively authors of all referred studies suggested, that elevated drug’s efficacy is the result of reduced pathological consequences of infection, and stimulation of immunity. Though, the direct interference with enzymatic systems of flavonoids on parasite in vivo is also possible. In general, co-administration of examined flavonoids with anthelmintic drugs seems to be the safe and effective way to potentiate therapy and reduce pathology at infections induced by various developmental stages of cestodes and trematodes.

Conclusion

In recent years, research focused on neglected tropical diseases where the infections caused by flatworm species also belong, has undergone a significant development. Nevertheless, a new chemical entities approved for treatment are still absent. Polyphenols are group of high interests. It is due to their low toxicity on the hosts and multiple mechanisms by which they can modulate pathologically changed processes during infections. Present review based on recently published findings highlights the potential of selected compounds: curcumin, genisten, quercetin and silymarin’s flavonolignans as sources of prospective molecules for drug development. These lipophilic molecules act primarily on soft tegument of flatworms where they can modulate structure and functions of ion channels, receptors and enzymes, and thus lead to the physiological imbalance or death of worms in vitro. Anthelmintic stress induced by transmembrane penetration leads to the disruption of energy metabolism, predominantly affecting glycogen stores and its degradation to the glucose. In addition, interference with other enzymatic systems involved in coordination of muscle activity is also proposed. The clear benefits of selected isoflavones as adjuvants to antiparasitic infection therapy support their potential in reduction of related pathology and modulation of the host immune responses. Despite the fact that multi-target activities of mentioned substances may represent a serious problem in the development.
of a very specific antiparasitic drugs a detailed discovery of the mode of their action as well as the development of new effective antiparasitic drugs with minimal side effects is a prospective and promising challenge for future research.

Conflict of Interest
Authors declare no conflict of interest.

Acknowledgements
This study was supported by the EU Structural Fund ITMS 2622020185 (MediPark), bilateral mobility project SAV-ACVR No. 18–24 and APVV project no. 17-0410 of the Ministry of Education, Science, Research and Sport of the Slovak Republic.

References
ABENAVOLI, L., IZZO, A. A., MILIC, N., CICALA, C., SANTINI, A., CAPASSO, R. (2018): Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmaceutical, and nutraceutical uses in liver diseases, Phytother Res., 32: 2202 – 2213, DOI: 10.1002/ptr.6171
ABOU EL DEHAB, M.M., SHAHAT, S.M., MAHMOUD, S.S.M., MAHAN, N.A. (2019): In vitro effect of curcumin on Schistosoma species viability, stemgul ultrastructure and egg hatchability. Exp. Parasitol., 199: 1 – 8. DOI: 10.1016/j.exppara.2019.02.017
ALLAM, G. (2009): Immunomodulatory effects of curcumin treatment on murine schistosomiasis mansoni. Immunobiology, 214(8): 712 – 727. DOI: 10.1016/j.imbio.2008.11.017
ANNAND, P., KUNNUMAKKA, A.B., NEWMAN, R.A., AGGARWAL, B.B. (2007): Bioavailability of curcumin: problems and promises. Mol. Pharm., 4(6): 807 – 18. DOI: 10.1021/mp700113r
ANDRES, S., PENNY, S., ZIEGENHAGEN, R., BAKHIYA, N., SCHAEFER, B.M., HIRSCH-ERNST, K.I., LAMPEN, A. (2018): Safety Aspects of the Use of Quercetin as a Dietary Supplement. Mol. Nutr. Food Res., 62(1): 1700447. DOI: 10.1002/mnfr.201700447
ANTHONY, K. P., SALEHI, M. A. (2013): Free Radical Scavenging and antioxidant activities of silymarin components. Antioxidants (Basel), 2(4): 398 – 407. DOI: 10.3390/antiox2040398
BISSON, J., MICALPINE, J. B., FRIESEN, J. B., CHEN, S. N., GRAHAM, J., PAULI, G. F. (2016): Can Invalid Bioactives Undermine Natural Product-Based Drug Discovery? J. Med. Chem., 59: 1671 – 1690. DOI: 10.1021/acs.jmedchem.5b00109
BOLLEN, M., KEPPENS, S., STALMANS, W. (1998): Specific features of glycogen metabolism in the liver. Biochem J., 336(Pt 1): 19 – 31. DOI: 10.1042/bj3360019
BAGUINE, C.G., BERTHANA, C.S., GONCALVES, U.O., MAlGALHAES, L.G., RODRIGUES, V., GMENES, V.M.M., GROPPO, M., SILVA, M.L.A.E., CUNHA, W.R., JANUARIO, A.H., PAULETTI, P.M. (2012): Schistosomical evaluation of flavonoids from two species of Styra against Schistosoma mansoni adult worms. Pharmacut. Biol., 50(7): 925 – 929. DOI: 10.3109/13880209.2011.649857
BRYANT, C., BEHM, A.C. (1989): Biochemical adaptation in parasites. London, Chapman and Hall, 259 pp.
BRZEZINSKI, A., DEBI, A. (1999): Phytoestrogens: the “natural” selective estrogen receptor modulators? Eur J Obstet Gynecol Reprod Biol., 85(1): 47 – 51. DOI: 10.1016/s0301-2115(98)00281-4
COURTNEY, A. (2017): The Ecstasy and Agony of Assay Interference Compounds, ACS Cent. Sci., 3: 143 – 147. DOI: 10.1021/acscentsci.7b00069
DAS, B., TANDON, V., SAHA, N. (2004): Effects of phytochemicals of Flemingia vestita (Fabaceae) on glucose 6-phosphate dehydrogenase and enzymes of gluconeogenesis in a cestode (Raillietina echinobothrida). Comp. Biochem. Physiol., 139(part C): 141 – 146. DOI: 10.1016/j.cjcpa.2004.10.004
DAS, B., TANDON, V., SAHA, N. (2006): Effect of isoflavone from Flemingia vestita (Fabaceae) on the Ca2+ homeostasis in Raillietina echinobothrida, the cestode of domestic fowl. Parasitol. Int., 55: 17 – 21. DOI: 10.1016/j.parint.2005.08.002
DAS, B., TANDON, V., SAHA, N. (2007): Genistein from Flemingia vestita (Fabaceae) enhances NO and its mediator (cGMP) production in a cestode parasite, Raillietina echinobothrida. Parasitology, 134(10): 1457 – 1463. DOI: 10.1017/S003118200700228X
DE PAULA AGUILAR, D., BRUNETTO MOREIRA MASCARDINI, M., REZENDE MORAIS, E., GRACIANO DE PAULA, R., FERREIRA, P.M., AFONSO, A., BELO, S., TOME OUCHIDA, A., CURTI, C., CUNHA, W.R., RODRIGUES, V., MAGALHãES, L.G. (2016): Curcumin generates oxidative stress and induces apoptosis in adult Schistosoma mansoni worms. PLoS ONE, 11(11): e0167135. DOI: 10.1371/journal.pone.0167135
DEHMLOW, C., ERHARD, J., DE GROOT, H. (1996): Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. Hepatology, 23(4): 749 – 754. DOI: 10.1053/jhep.1996.v23.pm000866328
DYER, J.I., KHAN, S.Z., BILMEN, J.G., HAWTIN, S.R., WHEATLEY, M., JAYED, M.U., MICHELANGEI, F. (2002): Curcumin: a new cell permeant inhibitor of the inositol 1, 4, 5 – triphosphate receptor. Cell calcium, 31(1): 45 – 52. DOI: 10.1054/ceca.2001.0259
EL-BAHY, N.M., BAZH, E.K.A. (2015): Anthelmintic activity of ginger, curcumin, and praziquantel against Raillietina cesticillus (in vitro and in vivo). Parasitol. Res., 114(7): 2427 – 2434. DOI: 10.1007/s00436-015-4416-0
EL-HAWARY, S.S., TAH, K.F., KIRILLOS, F.N., DAHAB, A.A., EL-MAHIS, A.A., EL-SAYED, S. H. (2018): Complementary effect of Capparis spinosa L. and silymarin with/without praziquantel on mice experimentally infected with Schistosoma mansoni. Helminthologia, 55(1): 21 – 32. DOI: 10.1515/helm-2017-0055
EL-LAKKANY, N.M., HAMMAM, O.A., EL-MAADAWY, E.H., BADAWY, A.A., AIIN-SHOKA, A.A., EBEID, F.A. (2012): Anti-inflammatory/anti-fibrotic effects of the hepatoprotective silymarin and the schistosomeside praziquantel against Schistosoma mansoni-induced liver fibrosis. Parasit. Vectors, 5: 9. DOI: 10.1186/1756-3305-5-9
EL-SAYED, N.M., FATHY, G.M., ABDEL-RAHMAN, S.A., EL-SHAFFEI, M.A. (2016): Cytokine patterns in experimental schistosomiasis mansoni infected mice treated with silymarin. J. Parasit. Dis., 40(3): 922
ESMAEILI, N., ANARIK, S.B., GHARAGOZLOO, M., MOAYEDI, B. (2017): Sil-lymarin impacts on immune system as an immunomodulator: One key for many locks. Int. Immunopharmacol., 50: 194 – 201. DOI: 10.1016/j.intimp.2017.06.030

FABRICANT, D.S., FARNISWORTH, N.R. (2001): The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 109 (1): 69 – 75. DOI: 10.1289/ehp.01109s169

GANAI, A.A., FAROOQI, H. (2015): Bioactivity of genistin: A review of in vitro and in vivo studies. Biomed pharmacother. 76: 30 – 38. DOI: 0.1016/j.biopharma.2015.10.026

GARCIA, H. H., MORO, P. L., SCHANTZ, P. M. (2007): Zoonotic hel-minth infections of humans: echinococcosis, cystercerosis and fascioliasis. Curr Opin Infect Dis, 20(5): 489 – 494

GÁSÁK, R., WALTEROVA, D., KREN, V. (2007): Sillybin and silymarin – new and emerging applications in medicine. Curr. Med. Chem. 14: 315 – 338. DOI: 10.2174/092986707779941159

GRAEFE, E.U., DERENDORF, H., VEIT, M. (1999): Pharmacokinetics and bioavailability of the flavonol quercetin in humans. Int. J. Clin Pharmacol. Ther., 37(5): 219 – 233

GUO, Y., BRUNO, R. S. (2015): Endogenous and exogenous mediators of quercetin bioavailability. J Nutr Biochem, 26(3): 201 – 210. DOI: 10.1016/j.jnutbio.2014.10.008

HALTON, D. (2004): Microscopy and the helminth parasite. Micron, 35(5): 361 – 390. DOI: 10.1016/j.micron.2003.12.001

HATCHER, H., PLANALP, R., CHO, J., TORTI, F.M, TORTI, S.V. (2008): Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci., 65(11): 1631 – 1652. DOI: 10.1007/s00018-008-7452-4

HAVSTEEN, B.H. (2002): The biochemistry and medical significance of flavonoids. Pharmacol. Ther. 96 (2 – 3): 67 – 202. DOI: 10.1016/s0163-7258(02)00298-x

HEIDARIAN, E., NOURI, A. (2019): Hepatoprotective effects of silymarin against diclofenac-induced liver toxicity in male rats based on biochemical parameters and histological study. Arch. Physiol. Biochem.:1 – 7. DOI: 10.1007/s13134-019.1620785

HEWITSON, P., IGNATOVA, S., YE, H., CHEN, L., SUTHERLAND, I. (2009): Intermittent counter-current extraction as an alternative approach to purification of Chinese herbal medicine, J chromatogr A, 1219(19): 4187 – 4192, DOI: 10.1016/j.chroma.2008.12.005

HRČKOVÁ, G., VELEBŇOVÁ, S. (2013): Pharmacological Potential of Selected Natural Compounds in the Control of Parasitic Diseases; Springer Science & Business Media: Berlin/Heidelberg, Germany, 115 pp.

HRČKOVÁ, G., MAČÁK KUBAŠKOVÁ, T., BENADO, O., KOFRONOVÁ, O., TUMOVÁ, L., BIEDERMANN, D. (2018): Differential effects of the flavono-lignans Silybin, Silychristin and 2,3-Dehydrosilybin on Mesocesto- tides vogae larvae (Cestoda) under hypoxic and aerobic in vitro conditions. Molecules (Basel, Switzerland) 23, article no. 2999. DOI: 10.3390/molecules23112999.

HRČKOVÁ, G., MAČÁK KUBAŠKOVÁ, T., MUDROŇOVÁ, D., BARDELČÍKOVÁ, A. (2020a): Concentration-dependent effect of silymarin on concanavalin A-stimulated mouse spleen cells in vitro. Eur. Pharmaceut. J. (in press). DOI: 10.2478/apfcu-2020-0003

HRČKOVÁ, G., MAČÁK KUBAŠKOVÁ, T., REITEROVÁ, K., BIEDERMANN, D. (2020b): Co-administration of silymarin elevates the therapeutic effect of praziquantel through modulation of specific antibody profiles, Th1/Th2/Tregs cytokines and down-regulation of fibrogenesis in mice with Mesocestoides vogae (Cestoda) infection. Exp. Parasitol., 213:107888. DOI: 10.1016/j.exppara.2020.107888

CHAMBERS, CH. S., HOLEČKOVÁ, V., PETRÁŠKOVÁ, L., BIEDERMANN, D., VALENTOVÁ, K., BUCHTA, M., KREN, V. (2017): The silymarin composi-tion... and why does it matter?? Food Res Int, 100: 339 – 353. DOI: 10.1016/j.foodres.2017.07.017

ISOHARY, H., Motojima, H., Onaga, S., Samet, I., Villarel, M.O, Han, J. (2014): Analysis of the erythroid differentiation effect of flavonoid apigenin on K562 human chronic leukemia cells. Chem. Biol. Interact., 220: 269 – 277. DOI: 10.1016/j.cbi.2014.07.00

JAVED, S., KOHLI, K., ALI, M. (2011): Reassessing Bioavailability of Silymarin, Altern Med Rev, 16(3): 239 – 249

KAHKHAE, K.R., MIRROSSENI, A., ALBADI, A., MOHAMMADI, A., JAVAD MOSAVI, M., HAFTCHESMEH, S.M., SATHYAPALAN, T., SEBEKAR, A. (2019): Curcumin: a modulator of inflammatory signaling pathways in the immune system. Inflammopharmacol., 27: 885 – 900. DOI: 10.1007/s10787-019-00607-3

KEUSER, J., UZINGER, J. (2005): Chemotherapy for major food-borne trematodes: a review. Expert Opin Pharmacother, 5(8): 1711 – 1726. DOI: 10.1517/146566666.5.8.1711

KAMEL, R.O. (2016): Interactions between mefloquine and the anti.fibrotic drug silymarin on Schistosoma mansoni infections in mice. J. Helminthol., 90(6): 760 – 765. DOI: 10.1017/ S0022149X16000018

KAR, P.K., TANDON, V., SAHA, N. (2002): Anthelmintic efficacy of Flemingia vestita: genistein-induced effect on the activity of nitric oxide synthase and nitric oxide in trematode parasite, Fasciolop­sis buski. Parasitol. Int., 51(3): 249 – 257. DOI: 10.1016/s1383-5769(02)00032-6

KAR, P.K., TANDON, V., SAHA, N. (2004): Anthelmintic efficacy of ge­nistein, the active principle of Flemingia vestita (Fabaceae): alterations in the free amino acid pool and ammonia levels in the fluke, Fasciolopsis buski. Parasitol. Int., 53: 287 – 291. DOI: 10.1016/j. parint.2004.04.001

KILCHMAN, F., MARCAIDA, M. J., KOTAK, S., SCHICK, T., BOSS, S. D., MAHENDRA, A., GONCZY, P., REYMOND, J.-L. (2016): Discovery of a selective aurora A Kinase Inhibitor by Virtual Screening. J Med Chem, 59(15):7188 – 211. DOI: 10.1021/acs.jmedchem.6b00709

KITA, K., HIRAIWA, H., MIYADERA, H., AMINO, H., TAKEO, S. (2002): Role of complex II in anaerobic respiration of the parasite mitochondria from Ascaris suum and Plasmodium falciparum, Biochim Biophys Acta, 1553: 123 – 139. DOI: 10.1016/s0005-2728(01)00237-7

KUHN, I., KELLENBERGER, E., SAAD-HASSANE, F., VILLA, P., ROGIANI, D., LOBSTEN, A., HAIECH, J., HIBERT, M., SCHUBER, F., MILLER-STEFFNER, H. (2010): Identification by high-throughput screening of inhibitors of Schistosoma mansoni NAD(+)-catalyzing enzyme. Bioorg. Med. Chem., 18: 7900 – 7910. DOI: 10.1016/j.bmc.2010.09.041
Lee, J.I., Narayan, M., Barrett, J.S. (2007): Analysis and comparison of active constituents in commercial standardized silymarin extracts by liquid chromatography-electrospray ionization mass spectrometry. J. Chromatogr. B. Anal. Technol. Biomed. Life Sci., 845(1): 95 – 103. DOI: 10.1016/j.jchromb.2006.07.063

Lee, K.H. (2010): Discovery and development of natural product-derived chemotherapeutic agents based on a medicinal chemistry approach. J. Nat. Prod., 73(3): 500 – 513. DOI: 10.1021/np900821e

Li-Webber, M. (2009): New therapeutic aspects of flavones: The anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Caicadin. Cancer Treat. Rev., 35(1): 57 – 68. DOI: 10.1016/j.ctrv.2008.09.005

Luz, P.P., Magalhães, L.G., Pereira, A.C., Cunha, W.R., Rodrigues, V., Andrade, E., Silva, M.L. (2012): Curcumin-loaded into PLGA nanoparticles: preparation and in vitro schistosomical activity. Parasitol. Res., 110 (2): 593 – 598. DOI: 10.1007/s00436-011-2527-9

Magalhães, L.G., Machado, C.B., Morais, E.R., Moreira, E.B., Soares, C.S., Da Silva, S.H., Da Silva F.A.A., Rodrigues, V. (2009): In vitro schistosomical activity of curcumin against Schistosoma mansoni adult worms. Parasitol. Res., 104(5): 1197 – 1201. DOI: 10.1007/s00436-008-1311-y

Mahmoud, M.S., Habb, F.S. (2003): Role of nitric oxide in host defense against Hymenolepis nana infection. J. Egypt. Soc. Parasitol., 33(2): 485 – 496

Marikovits, J., Linossier, C., Bosse, P., Courpie, J., Pierre, J., Jacques, M., Saillons, J., Saillons, M. (1989): Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res., 49(18): 5111 – 5117

Mata-Santos, H.A., Dutra, F.F., Rocha, C.C., Lino, F.G., Xavier, F.R., Chinala, L.A., Hossy, B.H., Castelo-Branco, M.T.L., Tedoro, A.J., Pavia, C.N., Dos Santos Pyrrho, A. (2014): Silymarin Reduces Proinflammatory Cytokines and reverses hepatic fibrosis in chronic murine Schistosomiasis. Antimicrob. Agents Chemother., 58(4): 2076 – 2083. DOI: 10.1128/AAC.01936-13

Mata-Santos, H.A., Lino, F.G., Rocha, C.C., Pavia, C.N., Castelo Branco, M.T., Pyrrho Ando, S. (2010): Silymarin treatment reduces granuloma and hepatic fibrosis in experimental schistosomiasis. Parasitol. Res., 107(6): 1429 – 1434. DOI: 10.1007/s00436-010-0214-8

Matsumoto, J., Sakamoto, K., Shinuyo, N., Kido, Y., Yamamoto, N., Yagi, K., Miyoshi, N., Nonaka, N., Katukura, K., Kita, K., Oku, Y. (2008): Anaerobic NADPH-Fumarate Reductase System Is Predominant in the Respiratory Chain of Echinococcus multilocularis, Providing a Novel Target for the Chemotherapy of Alveolar Echinococcosis, Antimicrob. Agents Chemother., 50(1): 164 – 170, DOI: 10.1128/AAC.00378-07

Mattey, D.L., Davies, P.T., Nixon, N.B., Slater, H. (1997): Transforming growth factor beta 1 and interleukin 4 induced alpha smooth muscle cell actin expression and myofibroblast-like differentiation in human sphenoidal fibroblasts in vitro: modulation by basic fibroblast growth factor. Ann. Rheum. dis. 56(7): 426 – 431. DOI: 10.1136/ard.56.7.426

Middleton, E.Jr., Kandaswami, C., Theoharides, T.C. (2000): The effects of plant flavonoids on mammalian cells: Implications for Inflammation, Heart disease, and cancer. Pharmacol. Rev., 52(4): 673 – 751

Moncada, S., Palmer, R.M.J., Higgs, E.A. (1991): Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev., 43(2): 109 – 142

Morais, E.R., Oliveira, K.C., Magalhães, L.G., Moreira É.B.C., Verulovski-Almeida S., Rodrigues, V. (2013): Effects of curcumin on parasite Schistosoma mansoni: A transcriptoric approach. Mol. Biochem. Parasitol., 187: 91 – 97. DOI: 10.1016/j.molbiopara.2012.11.006

Moskovitz, I.P., Rothman, J.H. (1996): Lin-12 and glp-1 are required zygotically for early embryonic cellular interactions and are regulated by maternal GLP-1 signaling in Caenorhabditis elegans. Development, 122(12): 4105 – 4117

Murata, K., Terao, J. (2015): Antioxidative flavonoid quercetin: implication of its intestinal absorption and metabolism. Arch. Biochem. Biophys. 417(1): 12 – 17. DOI: 10.1016/s0003-9861(03)00284-4

Naguleswaran, A., Spicher, M., Vonlaufen, N., Ortega-Mora, L.M., Torgerson, P., Gottstein, B., Hemphill, A. (2006): In vitro metacestodical activities of genisteen and other isoalloflavones against Echinococcus multilocularis and Echinococcus granulosus. Anti-microb. agents chemother., 50(11): 3770 – 3778. DOI: 10.1128/AAC.00578-06

Nelson, D.L., Cox, M.M. (2000): Lehninger’sprinciples of biochemistry. 3rd Edition, New York, Worth Publications. 1152 pp.

Oyeyemi, O., Adegbeyeni, O., Oyeyemi, I., Meena, J., Panda, A. (2018): In vitro ovicidal activity of poly lactic acid curcumin-nisin co-entrapped nanoparticle against Fasciola spp. eggs and its reproductivity. J. Basic Clin. Physiol. Pharmacol., 29(1): 73 – 79. DOI: 10.1515/jbcpp-2017-0045

Otero, L., Bonilla, M., Protasio, A.V., Fernández, C., Gladyshev, V. N., Salinas, G. (2010): Thioredoxin and glutathione systems differ in parasitic and free-living platyhelminths, BMC Genomics, 11: 237, DOI: 10.1186/1471-2164-11-237

Paranagama, P., Sakamoto, K., Amino, H., Akano, M., Miyoshi, H., Kita, K. (2010): Contribution of the FAD and quinone binding sites to the production of reactive oxygen species from Ascaris suum mitochondrial complex II, Mitochondrion, 10(2): 158 – 165. DOI: 10.1016/j.mito.2009.12.145

Pereira, C.A.J., Oliveira, L.L.S., Coaglio, A.L., Santos, F.S.O., Ceyar, R.S.M., Mendes, T., Oliveira, F.L.P., Conceensa, G., Lima, W.S. (2016): Anti-helminthic activity of Monomorica charantia L. against Fasciola hepatica eggs after twelve days of incubation in vitro. Vet. Parasitol., 228: 160 – 166. DOI: 10.1016/j.vetpar.2016.08.025

Pyszyskova, M., Biler, M., Bedermann, D., Valenta, R., Kuzma, M., Virba, J., Ulrichova, J., Sokolova, R., Mozovic, M., Popovic-Bujevic, A., Kubala, M., Trouillas, P., Kren, V., Vacek, J. (2016): Flavonolignan 2,3-dehydroderivates: Preparation, antiradical and cytotoxicity activity, Free Radic Biol Med, 90: 114 – 125, DOI: 10.1016/j.
freeradiobiomed.2015.11.01
Rao, H.S.P., Reddy, K.S. (1991): Isoflavones from Flemingia vesti
ta. Fitoterapia 63: 458
Rehman, A., Ullah, R., Gupta, D., Khan, M. A. H., Rehman, L., Beg,
M. A., Khan, A. U., Abidi, S. M. A. (2020): Generation of oxidative
stress and induction of apoptotic like events in curcumin and thy-
moquinone treated adult Fasciola gigantica worms, Exp Parasitol,
209: 107810. DOI: 10.1016/j.exppara.2019.107810
Reynaud, J., Guleit, D., Terreux, R., Lussignol, M., Walchshofer,
N. (2005): Isoflavonoids in non-leguminous families: an update.
Nat. Prod. Rep., 22: 504 – 515
Roberts, L.S. (1983): Carbohydrate metabolism. In Arme C, Pappas
P.W. (Eds) Biology of the eucestoda. New York, USA: Aca-
demic, pp. 343 – 390
Rolo, A.P., Oliveira, P.J., Morena, A.J., Palmeira, C.M. (2003): Pro-
tection against post-oschmic mitochondrial injury in rat liver by
silymarin or TUDC. Hepatol. Res., 26(3): 217 – 224. DOI: 10.1016/s
1386-6346(03)00108-6
Sakai, Ch., Tomitsuka, E., Esumi, H., Harada, S., Kita, K. (2012): Mit-
ochondrular fumarate reductase as a target of chemotherapy: from
parasites to cancer cells. Biochim Biophys Acta, 1820: 643 – 651,
DOI: 10.1016/j.bbadis.2011.12.013
Saller, R., Brignoli, R., Meier, J., Meier, R. (2008): An up-
dated systematic review with meta-analysis for clinical evidence of silymarin. Forsch. Komplement., 15(1): 9 – 20. DOI:
10.1159/000113648
Schuffenhauer, A., Varin, T. (2011): Rule-Based Classification of
Chemical Structures by Scaffold. Mol. Inf., 47: 646 – 664. DOI:
10.1002/minf.201100078
Sethi, N., Kang, Y. (2011): Notch signalling in cancer progression
and bone metastasis. Br. J. Cancer, 105(12): 1805 – 1810. DOI:
10.1038/bjc.2011.497
Sharma, R.A., Euden, S.A., Platten, S.L., Cooke, D.N., Shafiyat,
A., Hewitt, H.R., Marczylo, T.H., Morgan, B., Hemingway, D., Plum-
mer, S.M., Pirromheahed, M., Gescher, A.J., Steward, W.P. (2004):
Phase I clinical trial of oral curcumin biomarkers of systemic activ-
ity and compliance. Clin. Cancer Res., 10(20): 6847 – 6854. DOI:
10.1158/1078-0432.CCR-04-0744
Shehzad, A., Qureshi, M., Anwar, N.M., Lee, Y.S. (2017): Multifunc-
tional curcumin mediate multitherapeutic effects. J. Food Sci.,
82(9): 2006 – 2015. DOI: 10.1111/1750-3841.13793
Shoop, W. L., Mrozik, H., Fisher, M. H. (1995): Structure and activity
of avermectins and milbemycins in animal health. Vet Parasitol,
59(2): 139 – 156. DOI: 10.1016/0304-4017(94)00743-v
Smith, J.D., McManus, D.P. (1989): The physiology and biochemistry
of cestodes. Cambridge University Press, Cambridge. 398 pp.
Sobhy, M.M.K., Mahmoud, S.S., El-Sayed, S.H., Rozik, E.M.A., Raaf-
fat, A., Negm, M.S.I. (2018): Impact of treatment with a Protein
Tyrosine Kinase Inhibitor (Genistein) on acute and chronic exper-
imental Schistosoma mansoni infection. Exp. Parasitol., 185: 115
– 123. DOI: 10.1016/j.exppara.2018.01.013
Surai, P.F. (2015): Silymarin as a natural antioxidant: an overview
of the current evidence and perspectives. Antioxidants, 4(1): 204
– 247. DOI: 10.3390/antiox4010204
Szekanecz, Z., Koch, A.E. (2007): Macrophages and their products
in rheumatoid arthritis. Curr. Opin. Rheumatol., 19(3): 289 – 295.
DOI: 10.1097/BOR.0b013e32805e87ae
Tandon, V., Pal, P., Roy, B., Rao, H.S., Reddy, K.S. (1997): In vitro
anthelmintic activity of root-tuber extract of Flemingia vestita,
a indigenous plant in Shillong, India. Parasitol. Res. 83(5): 492 –
498. DOI: 10.1007/s004360050286
Tandon, V., Biyavadh, D., Saha, N. (2003): Anthelmintic efficacy of
Flemingia vestita (Fabaceae): effect of genistein on glycogen me-
tabolism in the cestode, Raillietina echinobothrida. Parasitol. Int.,
52(2): 179 – 183. DOI: 10.1016/s1383-5769(03)00006-0
Tandon, V., Das, B. (2007): In vitro testing of anthelmintic efficia-
cy of Flemingia vestita (Fabaceae) on carbohydrate metabolism in
Raillietina echinobothrida. Methods, 42(4): 330 – 338. DOI:
10.1016/j.ymeth.2007.01.005
Tandon, V., Das, B. (2018): Genistein: is the multifarious botanical
a natural anthelmintic too? J. Parasit. Dis., 42(2): 151 – 161. DOI:
10.1007/s12639-018-0984-0
Tomitsuka, E., Kita, K., Esumi, H. (2012): An anticancer agent, pyr-
vinium pamoate inhibits the NADH-fumarate reductase system—a
unique mitochondrial energy metabolism in tumor microenviron-
ments. J Biochem, 152(2): 171 – 183. DOI: 10.1093/jb/mvs041
Tousson, E., Beltagy, D.M., Gadia, M.A., Al-bebehanni, B. (2013):
Expressions of P53 and CD68 in mouse liver with Schistosoma mansoni infection and the protective role of silymarin. Toxicol. Ind.
Health, 29(8): 761 – 770. DOI: 10.1177/0748233712442733
Ullah, R., Rehman, A., Zafeer, M.F., Rehman, L., Khan, Y.A., Khan,
M.A.H., Khan, S.N., Khan, A.U., Abidi, S.M.A. (2017): Anthelmintic
Potential of thymoquinone and curcumin on Fasciola gigantica.
Plos One, 12(2): e0171267. DOI: 10.1371/journal.pone.0171267
Velebný, S., Hrková, G., Kogan, G. (2008): Impact of treatment with
praziquantel, silymarin and/or beta-glucan on pathophys-
ological markers of liver damage and fibrosis in mice infected with
Mesocestoides vogae (Cestoda) tetrathyridia. J. Helminthol.,
82(3): 211 – 219. DOI: 10.1017/S0022149X08960776
Velebný, S., Hrková, G., Königová, A. (2010): Reduction of ox-
idative stress and liver injury following silymarin and praziquantel
 treatment in mice with Mesocestoides vogae (Cestoda) infection.
Parasitol. Int., 59: 524 – 553. DOI: 10.1016/j.parint.2010.06.012
Wang, Y., Chen, S., Yu, O. (2015): Metabolic engineering of flav-
nonoids-rich Chinese bayberry (Mirica rubra Sieb. Et Zucc.) pulp
extracts on glucose consumption in human HepG2 cells. J. Funct.
Foods, 14: 144 – 153
Wang, W., Sun, C., Mao, L., Ma P., Liu, F., Yang, J., Qiao, Y. (2016):
The biological activities, chemical stability, metabolism and deliver-
systems of quercetin: a review. Trends Food Sci. Tech., 56:
21 – 38. DOI: 10.1016/j.lifs.2016.07.004
Wang, T.Y., Li, Q., Bi, K.S. (2018): Bioactive flavonoids in medi-
cinal plants: Structure, activity and biological fate. Asian J. Pharm.,
13(1): 12 – 23. DOI: 10.1016/j.ajps.2017.08.004
WeSolowska, O., Lania-Pietrzak, B., Kuzdzal, M., Stanczak, K., Mosiadz, D., Dobryszyncki, P., Ozyhar, A., Komorowska, M., Hendrich, A.B., Michalak, K. (2007): Influence of silybin on biophysical properties of phospholipid bilayers. Acta Pharmacol. Sin., 28(2): 296 – 306. DOI: 10.1111/j.1745-7254.2007.00487.x

YuAn, H., Ma, Q., Ye, L., Piao, G. (2016): The traditional medicine and modern medicine from natural products, Molecules, 21(5): 559. DOI: 10.3390/molecules21050559

Zhao, J., Agarwal, R. (1999): Tissue distribution of silibinin, the major active constituent of silymarin, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention, Carcinogenesis, 20(11): 2101 – 2108

Zhou, S., Hu, Y., Zhang, B., Teng, Z., Gan, H., Yang, Z., QunGwei, W., Huan, M., Mei, Q. (2008): Dose-dependent absorption, metabolism, and excretion of genistein in rats, 56(18): 8354 – 8359. DOI: 10.1021/jf801051d

Zhao, J., Agarwal, R. (1999): Tissue distribution of silibinin, the major active constituent of silymarin, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention, Carcinogenesis, 20(11): 2101 – 2108

Zhou, S., Hu, Y., Zhang, B., Teng, Z., Gan, H., Yang, Z., QunGwei, W., Huan, M., Mei, Q. (2008): Dose-dependent absorption, metabolism, and excretion of genistein in rats, 56(18): 8354 – 8359. DOI: 10.1021/jf801051d