Anti-inflammatory effect of Some Natural Flavonoids on the Hepatic Lysosomal Enzymes in Rats

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Abstract: The aim of this study was to evaluate the effect of different flavonoids such as: Quercetin, Rutin, Catechin, Gallic acid, Silymarin, Naringenin, Flavone, and Hisperetin by three concentrations "25, 50, and 100 µM/L" on the four markers lysosomal enzymatic activities in rat liver *in-vitro*. These enzymes are: Acid phosphatase "ACP"; β-galactosidase "β-GAL"; β-N-acetyl glucosaminidase "β-NAG", and β-GLU. Liver lysosomes were isolated by ultra cooling centrifugation at different speeds. The total activities and the release of the lysosomal enzymes were performed. The results revealed that the enzyme release of the four lysosomal enzymes appeared to significantly decrease ($P<0.05$) as compared to control under the effect of the three concentrations of each compound by different percentage values of inhibition. The protective effect of each flavonoid under investigation varied according to the concentration and the type of enzyme. It was observed that the low dose of each antioxidant compound exerted a highly percentage inhibition on the release of each lysosomal enzyme, while the high dose revealed a less inhibitory effect on the membrane permeability. This stabilizing effect was dose dependent. The medium concentration appeared to be moderate inhibitory effect. Also, the enzyme activity varied according to test-compared; Quercetin and Rutin which appeared to be more potent on the activities of β-GLU, β-GAL then β-NAG and ACP, while Catechin and Gallic acid were more potent on the activity of β-NAG and less potent on ACP activity. It was concluded that the most potent inhibitory effect was observed for Quercetin then Rutin and Silymarin and Naringenin, while the lowest inhibitory effect was observed for flavones and Hisperetin. As well as, this inhibitory effect on the lysosomal enzymes was dose and type-dependent.

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1. Introduction:

Lysosomes are bounded by a single, semi-permeable membrane and are responsible for the degradation of certain components that become obsolete for the cell or organism (Campbell and Smith, 2001). Most of the diseases result from deficiencies in single lysosomal enzymes, for example, Gaucherie's disease (the most common of these disorders) results from a mutation in the gene that encode a lysosomal enzyme required for the breakdown of glycolipids (Cooper, 2000).

The major lysosomal enzymes according to their importance as liver lysosomal markers are: Acid phosphatase, β-galactosidase, β-N-acetylglucosaminidase and β-glucuronidase (Sheeler and Bianchi, 1987). In many pathological conditions, changes in the state of lysosomes take place. The loss of the stability of lysosomal membrane has been observed in the leakage of enzymes from lysosomes (Stvolinskaya et al., 1992).

Antioxidants have a variety of biological effects in numerous mammalian systems in-vitro as well as in-vivo, free radical scavengers; antioxidants; pro-or antimutagens; anti-inflammatory, and antiviral or purgative effects. Some of them have been noted for their beneficial effect on cardiovascular diseases and cancer prevention (Middleton et al., 2000 and Gradolatto et al., 2004). The dietary antioxidants such as: Vitamin E, C, flavonoids and carotenoids appeared to be important in delaying and preventing certain human diseases, especially cardiovascular diseases and some types of cancer (Jacob and Burri, 1996).

Flavonoids are unusually large group of naturally occurring phenolic compounds, formed in plants from the aromatic amino acids, phenylalanine, tyrosine and acetate units to form the cinnamoyl structure of the flavonoid (Santos et al., 1998). Flavonoids are a large group of polyphenolic compounds that comprise an important class of secondary metabolites in plants. Their chemical structure is based on the phenylchromane or flavones ring system (Paladini et al., 1999).

A number of flavonoids are reported to possess anti-inflammatory activity. Hisperidin, a citrus flavonoid possesses significant anti-inflammatory and analgesic effects (Shahidi et al., 1998). Recently, apigenin, luteolin and quercetin have been reported to exhibit anti-inflammatory activity. Quercetin; Gallic acid ethyl ester and some as yet unidentified flavonoids might account for the anti-nociceptive action. The scavenging activity of flavonoids has been reported to be in the

order: Quercetin> Naringenin> Catechin> Flavones (Ratty, 1988 and Raj-Narayana et al., 2001).

Quercetin and other flavonoids are effective inhibitors of O_2^- production by cells and it is the most common native flavonoids occurring mainly in glycosidic forms such as Rutin (Nakamura et al., 2000). It can suppress lipid peroxidation in several biological systems, such as mitochondria and erythrocytes (Blackburn et al., 1987).

Rutin exhibits multiple pharmacological activities including antibacterial, antitumor, anti-inflammatory, anti-diarrhea, antiulcer, antimutagenic, myocardial protecting, vasodilator, immunomodulator and hepatoprotective activities (Kim et al., 2005). Rutin is a flavonoid "3,3',4',5,7-pentahydroxy flavones-3-rutinoside" (O'Neil et al., 2001). Rutin is hydrolyzed into quercetin and rutinose by enzymatic hydrolysis using rhamno-diastase enzyme from the seed of Rhamnus utilize (Sweetman et al., 2007).

Catechins, which are phenolics abundant in green tea, possess the antioxidative and pro-oxidative properties of Ca^{2+}-induced LDL oxidation. Catechins served as accelerators of oxidation (Yamanaka et al., 1997).

Gallic acid is commonly used in the pharmaceutical industry. It's seems to have anti-fungal anti-viral properties. Gallic acid act as antioxidant and helps to protect the cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells (Fiuza, 2004). The bioactivity of phenolics may be related to their antioxidant behavior.

Naringenin (NRG) is a molecule belonging to the class of flavanones and it is largely studied for its antioxidant activity, protective effect against the lipid peroxidation and hypolipidemic (Ubeaud et al., 1999). NRG was found to be a potent inhibitor of the isoforms (CYP1A, CYP3A) of human CYP450 in-vitro experiments (Tommasini et al., 2004).

Silymarin also inhibits the production of inflammatory mediators, such as necrosis factor α (TNF α) and nitric oxide and thus reduces damage to dopaminergic neurons (Kren and Walterova, 2005). The well known antioxidant properties of Silymarin can participate in its anti-inflammatory activity. The antioxidant activity of Silymarin and Silybin can also act as specific inhibitors of intestinal bacterial β-glucuronidase (Kim et al., 2005).

The aim of this study was to investigate the effect of some flavonoid compounds on the total enzymatic activities and the enzyme release of four lysosomal enzymes "ACP, β-GAL, β-NAG, and β-GLU in rat liver for looking for the stabilizing or labializing effect of these compounds on the lysosomal membrane.

2. Material and Methods

Test Compounds

Quercetin; Catechin; Rutin; Gallic acid; Silymarin; Naringenin; Flavone and Hisperetin were purchased from Fluka and Sigma USA in a purified form. All chemicals were supported in analytical form. Three graded concentrations were used from each antioxidant compound according to Janssen et al. (1998).

Quercetin; Rutin; Catechin, and Gallic acid were used by the concentration of 25, 50, and 100 μM/L, which equivalent (0.9, 1.7, 3.4 mg/ml for Quercetin); (1.66, 3.32, 6.65 mg/ml for Rutin); (0.73, 1.45, 2.90 mg/ml for Catechin), and (0.5, 0.9, 1.9 mg/ml for Gallic acid), as well as, Silymarin; Naringenin, Flavone and Hisperetin were used by the same μM/L which equivalent to (0.063, 0.126, 0.252 mg/ml for Silymarin); (0.68, 1.36, 2.72 mg/ml for Naringenin); (0.556, 1.111, 2.222 mg/ml for Flavone), and (0.756, 1.51, 3.02 mg/ml for Hisperetin). All compounds were dissolved in water using Tween 80 as an emulsifying agent.

Enzyme substrates

p-nitrophenyl phosphate (sodium salt) was used for acid phosphatase [EC.3.1.3.2].

p-nitrophenyl-ß-D-galactopyranoside was used for ß-galactosidase [EC.3.2.1.23].

p-nitrophenyl-2-acetamido-2-deoxy- ß-D-glucopyranoside was used for N-Acetyl- ß-glucosaminidase [EC.3.2.1.30].

phenolphthalein glucuronic acid (sodium salt) was used for ß- glucuronidase [EC.3.2.1.31]

All these substrates were purchased from Sigma Chemical Co. U.S.A.

Preparation of lysosomal fraction

Male albino rats weighing about 150-200g were used. After decapitation and bleeding, the liver was perfused in situ with 0.25 M ice-cold sucrose medium via portal vein at a rate of approximately 15 ml/minute according to the method of Tanaka and Iizuka (1968). The tissue was cutting into small pieces and dispersed in 0.25 M sucrose buffer pH (7.4) placed in CAT (R18) homogenizer. After homogenization, the volume was adjusted to 6.0 ml sucrose buffer 0.25 M contains 1.0g wet tissue of liver.

Incubation of lysosomes with antioxidant compounds

Incubation mixtures consisted of 1.0 ml of lysosomal fraction and 1.0 ml of antioxidant solution, the total volume was completed to 3.0ml by the addition of sucrose buffer solution. The tubes were incubated in a shaking water bath at 37°C/30min., Tubes of each antioxidant concentration were removed and centrifuged at 19000 r.p.m./15 min. The resulting supernatant was subjected to enzyme assay to determine the

activity of released enzymes (Robin and William, 1978).

Methods of enzyme assay

For determination the total enzymatic activity, some culture tubes containing 1ml antioxidant compounds+1ml lysosomal fraction+1ml TritonX-100 (0.1%) were exposure to thawing and freezing for three times, then centrifuged at 19000r.p.m/15min. The resulting supernatant was also subjected to each enzyme assay for determination the total enzymatic activities of each by lysosomal enzyme. The activities were measured spectrophotometerically according to the method of Van Hoof and Hers (1968) with slight modifications described by Younan and Rosleff (1974).

Statistical analysis of the results

All values are mean ± S. E. obtained from eight animals. For statistical analysis, one way ANOVA (Steal and Torry, 1980) with Duncan's variance (SPSS 10) was used to compare groups. In all the cases a difference was considered significant when ρ was < 0.05.

3. Results

The present results is a trial to illustrate some characteristics of the four marker hepatic lysosomal enzymes: Acid phosphatase "ACP"; N-acetyl-β-D- glucosaminidase "β-NAG"; β-galactosidase "β-GAL" and β-glucuronidase "β-GLU" under the effect of some flavonoid compounds such as: Quercetin; Rutin; Catechin; Gallic acid; Silymarin; Naringenin; Flavone, and Hisperetin, at three concentrations.

3.1. Effect of Quercetin; Rutin; Catechin, and Gallic acid on the lysosomal enzymatic activities

The effect of Quercetin and Rutin by the three concentrations under investigation on the lysosomal enzyme releases after 30min. of incubation was observed in Table (1). It appears to have stabilizing effect on the membrane permeability of lysosomes. This stabilizing effect of the two flavonoids may be due to the activity of these compounds on the membrane permeability as anti-inflammatory.

As indicated from the results by quercetin, the highest effect of the stabilization on the four lysosomal enzymes appeared for β-GAL and β-GLU, then ACP, and β-NAG. The higher concentration of quercetin revealed a higher activity for the four lysosomal enzymes, while the lowest concentration exerted thelowest enzyme activity. Also, *Rutin* revealed an inhibitory effect according to the concentration levels, the highest activity was observed for β-GLU then β-GAL and β-NAG, while the lowest enzymatic activity was observed for ACP.

The results indicated that the effect of these flavonoid compounds on the lysosomal enzymeatic activities of these marker enzymes approved to be enzyme and concentration dependent, the total enzymatic activity appeared to have high values for ACP activity, then β-GAL and β-NAG. The lowest activity was observed for β-NAG. This may be due to the enzyme synthesis by rough endoplasmic reticulum.

3.2. Effect of Silymarin; Naringenin; Flavone, and Hisperetin on the lysosomal enzymatic activities

The results in Table (2) revealed that these compounds showed a different inhibitory effect by variable percentage of inhibition according to the concentration levels of the compounds "25, 50, 100μM/L" and the enzyme type. It is revealed the enzyme release appeared to be varied by different percentage of release. ACP, β-GAL, β-NAG exerted a stabilizing effect under the effect of the four compounds by the three concentrations with the percentage variable.

The low concentration of each flavonoid compound exerted a less stabilizing effect, while the high concentration level revealed a highly stabilizing effect. β-glucuronidase appeared to be variable response according to the compound and concentration level. The total enzymatic activity of β-GLU and β-Glactosidase approved to be highly activity, then ACP and β-NAG.

Silymarin appeared highly stabilizing effect on β-GAL and ACP by variable of percentage inhibition dependent on the concentration of compound as compared to control group. The low dose exerted a highly percentage, while the high dose appeared to be less inhibitory effect.

Naringenin, flavones, and Hisperetin exerted a variable inhibitory percentage according to the concentration and the enzyme type. NRG exerted a highly inhibitory effect on β-GAL and β-GLU activities, then ACP, and β-NAG. Flavone and Hisperetin revealed a most potent inhibitory effect on β-GAL then β- GLU, the lowest inhibitory effect was appeared to be for ACP and β-NAG.

Table 1. Effect of (Qu, Quercetin; Ru, Rutin, CAT, Catchin, and Gal, Gallic acid) at three concentrations (LD, Low Dose, 25, MD, Medium dose, 50, and HD, High Dose, 100 µM/L) on the four marker lysosomal enzymatic activities (ACP, Acid phosphatase ; β-GAL, β-Galactosidase; β-NAG, β -N-acetyl glucosaminidase and β-GLU, β-Glucuronidase) in rat liver lysosomes after 30 minutes of incubation. (n=8).

Treatments	The lysosomal enzymatic activities (nmole/ml/hr)			
	ACP	β-GAL	β-NAG	β-GLU
Total activities	13968.33±0.04 ↑140%*	2207.41±0.06 ↑53%*	7096.84±0.07 ↑35%*	1916.73±0.03 ↑40%*
Control	5799.98±0.09 ---------	1438.80±0.05 --------	5271.90±0.04 ----------	1365.97±0.04 --------
Qu				
LD	2660.51±0.03 ↓54%*	315.74±0.03 ↓78%*	2352.28±0.04 ↓55%*	118.83±0.04 ↓91%*
MD	2926.30±0.03 ↓50%*	350.93±0.04 ↓76%*	2402.81±0.07 ↓54%*	136.38±0.04 ↓90%*
HD	3357.88±0.04 ↓42%*	363.89±0.04 ↓75%*	2656.49±0.08 ↓50%*	226.55±0.04 ↓83%*
Ru				
LD	3344.72±0.04 ↓42%*	501.85±0.05 ↓75%*	2176.14±0.07 ↓59%*	223.74±0.04 ↓84%*
MD	3368.40±0.03 ↓42%*	514.35±0.04 ↓64%*	2329.12±0.03 ↓56%*	294.97±0.04 ↓78%*
HD	3694.72±0.03 ↓36%*	546.30±0.04 ↓62%*	2620.00±0.10 ↓50%*	349.01±0.04 ↓74%*
CAT				
LD	3673.67±0.04 ↓37%*	987.50±0.04 ↓31%*	2914.39±0.06 ↓45%*	965.15±0.05 ↓29%*
MD	5057.87±0.06 ↓13%†	1075.00±0.03 ↓25%*	3400.70±0.04 ↓36%*	1067.25±0.04 ↓22%*
HD	5373.63±0.04 ↓7%	1232.87±0.03 ↓14%†	4170.53±0.04 ↓21%*	1145.50±0.03 ↓16%†
Gal				
LD	3936.82±0.02 ↓32%*	462.04±0.05 ↓68%*	919.30±0.17 ↓83%*	473.57±0.04 ↓65%*
MD	4823.66±0.04 ↓17%†	819.44±0.03 ↓43%*	2552.90±0.05 ↓52%*	1085.85±0.04 ↓21%*
HD	5239.44±0.10 ↓10%†	1257.87±0.03 ↓13%†	4552.98±0.04 ↓14%†	1106.55±0.08 ↓19%†

Each value in the table was obtained by calculating the average of eight experiments ± standard deviation, The various superscript letters indicate statistically significant differences in the Duncan test, with $P < 0.05$,*† **:** Insignificant at P> 0.05.

Table 2. Effect of [SIL, Silymarin; NRG, Nargenin; Fla, Flavone, and His, Hispertin] at three concentrations (LD, Low Dose, 25, MD, Medium dose, 50, and HD, High Dose, 100 µM/L) on the four marker lysosomal enzymatic activities (ACP, Acid phosphatase ; β-GAL, β-Galactosidase; β-NAG, β -N-acetyl glucosaminidase and β-GLU, β-Glucuronidase) in rat liver lysosomes after 30 minutes of incubation. (n=8).

Treatments	The lysosomal enzymatic activities nmole/ml/hr			
	ACP	β-GAL	β-NAG	β-GLU
Total activities	12905.20±0.05 ↑117%*	1997.83±0.05 ↑83%*	5406.73±0.04 ↑71%*	663.16±0.02 ↑194%*
SIL				
LD	3048.93±0.02 ↓49%*	148.51±0.01 ↓86%*	2005.66±0.01 ↓37%*	127.37±0.01 ↓43%*
MD	3214.07±0.02 ↓46%*	275.34±0.02 ↓75%*	2168.20±0.03 ↓31%*	136.53±0.01 ↓39%*
HD	3623.85±0.01 ↓39%*	353.39±0.02 ↓68%*	2611.16±0.11 ↓17%†	149.47±0.01 ↓34%*
NRG				
LD	3131.50±0.04 ↓47%*	165.85±0.01 ↓85%*	1967.89±0.05 ↓38%*	115.44±0.01 ↓49%*
MD	4477.06±0.03 ↓25%*	406.50±0.02 ↓63%*	2539.76±0.10 ↓20%*	119.82±0.01 ↓47%*
HD	5400.61±0.02 ↓9%†	539.84±0.01 ↓50%*	2606.73±0.03 ↓18%†	158.95±0.01 ↓29%*
Fla				
LD	3834.86±0.02 ↓35%*	224.39±0.01 ↓79%*	2194.95±0.11 ↓31%*	123.16±0.01 ↓45%*
MD	4899.08±0.02 ↓18%†	368.02±0.02 ↓66%*	2464.83±0.05 ↓22%*	127.54±0.01 ↓43%*
HD	5519.88±0.04 ↓7%†	565.85±0.02 ↓48%*	2975.54±0.03 ↓6%†	138.95±0.01 ↓38%*
His				
LD	3877.68±0.02 ↓35%*	205.96±0.01 ↓81%*	2139.14±0.02 ↓32%*	141.40±0.01 ↓37%*
MD	4397.55±0.01 ↓26%*	315.45±0.02 ↓71%*	2400.61±0.07 ↓24%*	154.91±0.01 ↓31%*
HD	5339.45±0.02 ↓10%†	476.96±0.01 ↓56%*	2705.81±0.08 ↓14%†	175.26±0.01 ↓22%*

Each value in the table was obtained by calculating the average of eight experiments ± standard deviation, The various superscript letters indicate statistically significant differences in the Duncan test, with $P < 0.05$,*† **:** Insignificant at P> 0.05.

4. Discussions

4.1. Effect of Quercetin; Rutin; Catechin, and Gallic acid on the lysosomal enzymatic activities

ACP has been considered as the marker enzyme of the hepatic lysosomes and for measurement of cell viability by virtue of its presence in surplus amounts not only in the secondary lysosomes but also in the primary lysosomes (Lin et al., 2000). The other lysosomal enzymes β-GAL; β-NAG and β-GLU are very

important for liver lysosomal functions (Vogler et al., 2005 and Cany et al., 2007).

The total enzymatic activity appeared to have high values for ACP activity, then β-GAL and β-NAG. The lowest activity was observed for β-NAG. This may be due to the enzyme synthesis by rough endoplasmic reticulum. Also, it may be to regulatory genetic coding which was accompanied by elevation in the enzymatic activities and stabilization mediated by prostaglandin synthetase (Hope and Welton, 1983 and Teleb et al., 1990). It was found the anti-inflammatory activities of the three flavonoids "Rutin; Quercetin and Hesperdin shows that Rutin was the most active flavonoid in the chronic phase (Guardia et al., 2001). A number of flavonoids are reported to possess anti-inflammatory activity, Hesperidin, a citrus flavonoid possesses significant anti-inflammatory effect. Recently Quercetin and Rutin have been reported to exhibit anti-inflammatory activity and acting as antioxidants exhibited as anti-inflammatory, antiviral, as well as, anticancer activity.

The scavenging activity of flavonoids has been reported to be in the order: Quercetin> naringenin> catechin> flavones which have an stabilizing effect (Robak and Glyglewski, 1988 and Raj-Narayana et al., 2001).

Teleb et al. (1998) mentioned that the biochemical functions of membrane together with their physiological properties are critically dependent on their phospholipid components. They observed that the activities of the enzyme seem to be dose-dependent and the differences in the activities may be due to the behavior of each enzyme towards the functional groups of each compound. The phenolic and hydroxyl groups could affect the enzyme activity and the membrane permeability (Caruso et al., 2006).

Also, Nakagawa et al. (2000) indicated that quercetin inhibited the release of hydrolases from lysosomal vesicles exposed to oxygen free radicals probably owing to the inhibition of oxidative damage of lysosomal membranes.

Hosni and Stenersen (2000) illustrated that the variation in the enzyme activity was dependent on the type of the enzyme; it may be depending on the enzyme kinetics and the behavior of each enzyme.

Quercetin is the deglycosylated product of Rutin. It has a potential to inhibit free radical process in cells by scavenging $O^{\cdot-}_2$, blocking lipid peroxyl radicals, reacting with peroxyl of lipid peroxyl radicals, inhibiting formation of $HO^{\cdot}$ and chelating iron ions. Also, it was observed to be cytotoxic in a dose-dependent manner. Cytotoxic may involve formation of $O^{\cdot-}_2$ or its metabolites O-quinone. Such species bind irreversibly to cell constituents by covalent binding with sulfhydryl groups or other essential groups (Metodiewa et al., 1999).

The study of Morikawa et al. (2003) indicated that the flavanols modulated the inflammatory response by modulating the prostavoid synthesis as well as cytokine production. Rotelli et al. (2003) found that Quercetin was the most compound in reducing paw edema induced by carrageenan.

The effect of this antioxidant compound showed a variety in the percentage change depends on the concentration and the type of the lysosomal enzyme of the tested concentrations Table (1). Catechin revealed a significant decrease on the lysosomal enzymatic activities as compared to control. The stabilizing effect of the Catechin was varied according to the enzyme and dose. The inhibitory effect appeared to be high at the low concentration and the inhibition effect was reduced at higher concentration. This effect was happened for ACP, β-GAL, β-NAG, and β-GLU activities.

Davila et al. (2002) suggested that Catechin (CAT) and Silybin (SIL) may act by stabilizing the plasma membrane against the toxic effect of hepatocytes cell injury induced by erythromycin. Also, Niebes and Ponard (2002) reported that Catechin exerts in-vivo stabilizing effect on lysosomal membranes in rat liver. The activity of ACP, β-GLU, and β-NAG was significantly reduced by 15-20% as compared to the control.

Concerning the effect of Gallic acid, the enzyme activity of the four lysosomal enzymes was varied according to the compound concentration and the type of enzyme. At the high dose of Gallic acid the enzyme activity was decreased by different values. The activities of these enzymes exerted a significantly inhibition by the dose-dependent.

Prince et al. (2009) investigate that Gallic acid prevents the lysosomal membrane damage against isoproterenol induced cardiac damage and this effect were due to anti-lipoperoxidative and antioxidant effects of gallic acid.

Ashcroft and Ashcroft (1992) and Ivan et al. (1999) found that the antioxidant compounds containing phenolic groups have main action on the membranes by their effect on Ca^{2+} channels and by increasing Ca^{2+}influx and causing a rise in cytosolic Ca^{2+}.

Abdel-Gawad et al. (2005) and Teleb et al. (1990) assumed that such changes in marker enzymes activities could be attributed to the variability in lysosomal membrane stabilization and labilization, which affects the outward leakage of these enzymes.

4.2. Effect of Silymarin; Naringenin; Flavone, and Hisperetin on the lysosomal enzymatic activities

It was investigated that Rutin, Quercetin (flavonols) and Hesperdin (flavanone) inhibited

both acute and chronic phases of inflammation; Rutin was the most active in the chronic phase (Guardia et al., 2001). Pretreatment with both Catechin and Silybin resulted in less enzyme leakage. It may act by stabilizing the plasma membrane against toxic insult (Davila et al., 2002).

Martin et al. (2006) found anti-ulcerogenic effect for the two flavonoids; quercetin and Naringenin, in acute gastric ulcer in a significant decrease as compared to control group

Hart et al. (1990) found that Hespertin and Luteolin inhibited myeloperoxidase (MPO) release. In addition to, quercetin by 200μM inhibited β-hexosaminidase synthesis as well as total culture-associated enzyme activity. Rutin, although less effective, the flavonoid effects were all concentration-dependent.

Hispertin inhibited receptor-mediated endocytosis of β-hexosaminidase by fibroblasts up to 50% of control uptake. Naringenin, Quercetin moderately inhibited uptake by 30%. The results demonstrate that certain naturally occurring flavonoids affect the secretion of lysosomal enzymes as well as their endocytosis by fibroblasts (Vladutiu and Middleton, 1986).

These results indicated that the effect of these flavonoids on the lysosomal permeability and the enzyme secretion through the lysosomal membrane appeared to be variable according to the enzyme type and dose-dependent.

In general, the in-vitro study showed that, addition of (25, 50 and 100μM/L) of Quercetin, Rutin, Catechin, Gallic acid, Silymarin, Naringenin, Flavone, and Hispertin to the lysosomal enzymes "ACP, β-GAL, β-NAG, and β-GLU" of rat liver exerted an inhibitory effect by different percentage values. It was suggested that flavones are strong inhibitors and flavonols such as quercetin are moderate inhibitors of the marker lysosomal enzymes and the cyclooxygenase. Also, the study suggested that glycosylated compounds are less potent inhibitors than their aglycones as indicated by (Grygleuski et al., 1987 and Karin-Janssen et al., 1998). The molecular bases of the anti-inflammatory and anti-carcinogenic effects of Silymarin are yet unknown; they might be related to inhibition of the transcription factor NF-KB, which regulates and coordinates the expression of various genes involved in the inflammatory.

Silymarin (accomplished by silybin glycosylation also suppressed the TNF-α-induced protein and mRNA expression of adhesion molecules. Silymarin also inhibited the TNF-α-induced activation of mitogen-activated protein kinase and caspase activation. The inhibition of the activation of NF-KB and the kinases may provide in part the molecular basis for the anti-inflammatory effects of silymarin and its effects on caspases may explain its role in cytoprotection (Kren and Walterova, 2005).

5. Conclusion and Recommendation

The effects of the plant natural flavonoids under investigation on the four marker lysosomal enzymatic activities were approved to be stabilizing effect on the membrane of lysosomes. This effect was varied according to the concentration of compound, the enzyme type and each flavonoid. The relative order of potency of tested flavonoids that we should use in our daily life was: Quercetin> Rutin> Silymarin> Catechin> Gallic acid> Naringenin> Flavone> Hispertin but with some precautions. Data raise the possibility that some of the beneficial effects as a protective action of flavonoids which rich in fruits and vegetables are related to the prevention of body from the inflammatory action in the form of stabilizing effect of these compounds on the lysosomal membrane permeability. Such stabilization of the lysosomes may have a beneficial effect in various hepatic disorders involving abnormal fragility of the lysosomes.

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