ORIGNAL ARTICLE

Effects of Crude Flavonoids from Ginger (*Zingiber officinale*), on Serum Uric Acid Levels, Biomarkers of Oxidative Stress and Xanthine Oxidase Activity in Oxonate-Induced Hyperuricemic Rats

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Abstract

Increased serum uric acid is known to be a major risk related to the development of several oxidative stress diseases. The aim of this study was to investigate the effect of crude flavonoids of Ginger (*Zingiber officinale*) extract on serum uric acid levels, xanthine oxidase (XO) activity and oxidative stress biomarkers in normal and oxonate-induced hyperuricemic rats. A total of 60 male Wistar rats were randomly divided into ten equal groups; including 5 normal groups (vehicle, flavonoids low dose, flavonoids medium dose, flavonoids high dose and allopurinol) and 5 hyperuricemic groups (vehicle, flavonoids low dose, flavonoids medium dose, flavonoids high dose and allopurinol). Flavonoids fraction at three different concentration (50.100 and 250µg/kg.b.w) and allopurinol (5mg/kg.b.w) administrated to the corresponding groups by oral gavages once a day for 4 weeks. The results showed that Ginger' s flavonoids constituents did not cause any significant reduction in the serum uric acid levels and xanthine oxidas activity in normal rats, but significantly reduced the serum uric acid levels and the enzyme activity of hyperuricemic rats in a time-dependent manner. Flavonoids treatment led also to a significant increase in total antioxidant capacity and decrease in malondialdehyde concentration in hyperuricemic rats. Although the hypouricemic effect of allopurinol was much higher than that of flavonoids as a possible alternative for allopurinol, or at least in combination therapy, it could not significantly change oxidative stress biomarkers more than Ginger' s flavonoids. These features of flavonoids make them to minimize the side effects of allopurinol to treat hyperuricaemia and oxidative stress diseases.

INTRODUCTION

Gout is a chronic metabolic disease with symptoms such as increased uric acid and tissue inflammation (1). Xanthine oxidase generating uric acid and oxidant is a key enzyme in tissue damage. This enzyme converts hypoxanthine and xanthine to uric acid (2). Xanthine oxidase using oxygen generates a high mount of free radicals and super oxide and so is a main source of generating super oxide ion and free radicals in body (3). Allopurinol is the only clinically used Xanthine oxidase inhibitor also having many side effects such as Hypersensitivity syndrome, Stevens Johnson syndrome and renal toxicity (5,6). It is necessary to develop compounds with Xanthine oxidase inhibitory activities which are devoid of the undesirable side effects of Allopurinol. A potential source of such compounds can be obtained from medicinal plants (4,6). Flavonoids and polyphenolic crude extracts have been reported to possess Xanthine oxidase inhibitory activity (7, 8) .

Ginger (*Zingiber officinale*) is one of the most widely spices worldwide. Besides its extensive use as a spice, the rhizome of ginger has also been used in traditional herbal medicine. The health-promoting perspective of ginger is often attributed to its rich photochemistry (9). Jolad *et al*. (2004) identified more than 60 compounds in fresh ginger grouped into two broader categories; volatiles and non-volatiles. Volatile compounds including sesquiterpene and mono terpenoids hydrocarbons providing the distinct aroma and taste of ginger and non-volatile compounds include gingerols, shogaols, paradols and zingerone (10).

 Ginger, as an antioxidant, improves diabetes induced oxidative stress and its complications through prevention of lipid peroxidation and protein oxidation (11). The rich phytochemistry of ginger includes components that scavenge free radicals produced in food chains or biological systems . Increased production of free radicals and reduction in cellular homeostasis results in oxidative stress that can lead to DNA damage (12).

Ginger is one of the richest sources of antioxidants that paved the way for its utilization to scavenge free radicals and allied health discrepancies, the active ingredients of ginger include gingerols, which exhibit antioxidant activity, and inhibit xanthine oxidase enzyme, that involved in the generation of reactive species (13, 14).

   The aim of the present research is finding out functional plant components of anti-gout activity. This is accomplished through testing different plant extracts as uric acid lowering, antioxidant and anti-inflammatory in experimental gout model in rats. Specific phytochemicals constituents of the studied plants have been assessed as well.

Materials and methods:
Extraction of flavonoids from ginger
   For preparation of crude flavonoids extract from Z. officinale, 1Kg of rhizome dried powder was extracted by cold maceration with 3 L of 70% Ethanol for 72 h at room temperature then the mixture filtered with Buchner funnel . The extraction was repeated twice to ensure all flavonoids were extracted. The combined filtrates were concentrated in a vacuum evaporator to afford a syrupy brown residue. This residue was suspended in 250 ml hot water (60°C), filtered, the filtrate placed in separation funnel and treated with petroleum ether (250 ml × 3) to remove fats. The aqueous portion was then separated, collected, and fractionated with N-butanol saturated water (250 ml × 3). The aqueous portion was discarded and the N-butanol portion was then separated, collected before being fractionated with 1% KOH. The KOH portion was then fractionated with dilute HCl (2%) and N-butanol saturated water. The dilute HCl portion was discarded. The N-butanol portion was then separated, collected, and dried to obtain a crude extract of flavonoids. Before use, the stock was further diluted in DMSO to give the final indicated concentrations (Elkady *et al.,*2014; Ibrahim *et al.,*2008).

Animal model of hyperuricemia in rats
   Experimentally-induced hyperuricemia in rats (due to inhibition of uricase with potassium oxonate) was used to study antihyperuricemic and antioxidant effects of test plant extracts .Briefly,250 mg/kg, uricase inhibitor, potassium oxonate (PO), dissolved in 0.9% saline solution was administrated intraperitoneally to each animal 1 h before oral administration of test compounds

Experimental design
   The animals were randomly divided into ten equal groups (n = 6). group 1: untreated, nonhyperuricemic animals; group 2: normal animals given 50 µg/kg flavonoids; group 3: normal animals given 100 µg/kg flavonoids; group 4: normal animals given 250 µg/kg flavonoids; group 5: normal animals given 5mg/kg allopurinol; group 6: hyperuricemic animals; group 7: hyperuricemic animals given 50 µg/kg flavonoids; group 8: hyperuricemic animals given 100 µg/kg flavonoids; group 9: hyperuricemic animals given 250 µg/kg flavonoids;; group 10: hyperuricemic animals given 5 mg/kg allopurinol.

Serum uric acid and xanthine oxidase activity was determined after   14and 28 days.At the end of the experiment, rats were sacrificed and the blood collected by transcardiac puncture 1 hour after administration of the test compound. Serum was obtained by centrifuging blood sample at 6000 rpm for 10 minutes, the serum stored at -20 °C until used.

Measurement of Malondialdehyde (MDA):
The level of malondialdehyde was determined by a modified procedure described by Guidet B. and Shah S.V.,(1989)(15). The principle of the test was based on the reaction of MDA with thiobarbituric acid (TBA); forming an $MDA-TBA_2$ product that absorbs strongly at 532 nm .

Measurement of Serum Glutathione Peroxidase (GPx_1):
The ELISA kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of human GP_X in serum, plasma and other biological fluids. The microtiter plate provided in this kit has been pre-coated with an antibody specific to GP_{X1}. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for GP_{X1}. Next, Avidin conjugated to Horseradish

Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain GP_{X1}, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of GP_{X1} in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Measurement of Catalase Activity (CAT): Bioassay Systems' improved assay directly measures catalase degradation of H_2O_2 using a redox dye. The change in color intensity at 570nm or fluorescence intensity (λ em/ex = 585/530nm) was directly proportional to the catalase activity in the sample.

Statistical analysis

Experimental data were analyzed using Graph Pad Prism 5.1 Software and results were presented as mean values mean ±SEM. The statistical evaluation of results were analyzed utilizing One-way variance analysis (ANOVA), followed by Tukey's test P value< 0.05 were considered statistically significant

Results:

Effects of flavonoids on serum uric acid level and XO activity *In vivo*

A time dependent study on Flavonoids were conducted to evaluate the effect of flavonoids on urate level during 28 day of the oral administration of flavonoids and allopurinol in hyperuricemia model and normal healthy rats. Results in Table 1 show that administration of crud flavonoids failed to score a significant effect in serum uric acid in normal rats after 14 and 28 days, but allopurinol, as a putative inhibitor of XO, significantly reduced ($P<0.05$) the levels of serum uric acid in the normal rats from the first day. In the hyperuricemic groups The administration of potassium oxonate significantly increase serum uric acid level in rats after 1 day. The administration flavonoids (250 and100 µg/ kg.b.w) significantly suppressed the elevation serum uric acid levels after 14 day, the reduction ratios after 28 day were 58.38 , 22.68 respectively. The high dose lower the uric acid by 1.898 ± 0.35 mg and the medium dose lower it by 0.737 ± 0.17 . The stander drug allopurinol lower the urate level down by 2.085 ± 0.19 mg with reduction ratio 65.55 %. The low dose of flavonoids administrated to rats had the lowest significant reduction ratio of uric acid production 7.17% after 28 day .

Table 1: Effect of the orally intake of flavonoids on serum uric acid levels (Mean ± SE mg/dl) in normal and hyperuricemia rats induced by potassium oxonate (a time dependent study).

Group	Day1	Day14	Day 28
Normal (vehicle)	1.99±0.33	2.00±0.24	2.1±0.23
Normal + Flavonoids 25µg/kg	1.98±0.30	2.10±0.26	2.02±0.31
Normal + Flavonoids 50µg/kg	2.05±0.28	2.03±0.25	2.06±0.27
Normal + Flavonoids 100µg/kg	2.10±0.27	2.05±0.29	2.01±0.32
Normal +Allopurinol	1.45±0.29[##]	1.33±0.36[###]	0.94±0.37[###]
Hyperuricemia	3.26±0.48[###]	3.22±0.47[###]	3.24±0.43[###]
Hyper. + Flavonoids 25µg/kg	3.25±0.45	3. 07±0.36	2.99±0.37*
Hyper. + Flavonoids 50µg/kg	3.24±0.43	2.56±0.32**	2.51±0.28***
Hyper.+ Flavonoids 100µg/kg	3.23±0.38	2.22±0.29***	1.35±0.45***
Hyperuricemic +Allopurinol	2.12±0.28***	1.46±0.38***	1.16±0.30***

* *P* < 0.05 when compared with hyperuricemia control group, ** *P* < 0.01 when compared with hyperuricemia control group, *** *P* < 0.001 when compared with hyperuricemia control group, [#]*P* < 0.05 when compared with vehicle control group, [###] *P* < 0.001 when compared with vehicle control group.

In a dose dependent study that conducted to evaluate the effect of flavonoids on serum xanthine oxidase activity , results (**Table 2**) show that in normal groups administration of flavonoids did not make a significant effect on XO activity , but Allopurinol did. In hyperuricemia groups treated with flavonoids (250 ,100 and 50µg/ kg.b.w) there were a significant reduction in serum XO activity by 62.30, 59.16 and 28.07% respectively.

Table 2: Results of orally intake of three different concentration of flavonoids on serum XO activity in normal and hyperuricemia rats induced by potassium oxonate (a dose dependent study)

Group	XO activity U/ml	XO inhibition %
Normal (vehicle)	1.32 ± 0.24	-
Normal + Flavonoids (50 µg/kg)	1.31 ± 0.68	0.75
Normal + Flavonoids (100 µg/kg)	1.29 ± 0.45	2.27
Normal + Flavonoids (250 µg/kg)	1.24 ± 0.33	6.06
Normal + Allopurinol	1.02 ± 0.52	22.72
Hyperuricemiaa	3.42 ± 0.12	-
Hyperuricemia + Flavonoids (50 µg/kg)	2.46 ± 0.07	28.07
Hyperuricemia + Flavonoids (100 µg/kg)	1.56 ± 0.11	59.16
Hyperuricemia + Flavonoids (250 µg/kg)	1.44 ± 0.33	62.30
Hyperuricemia +Allopurinol	1.2 ± 0.15	68.58

Effect of flavonoids on MDA and some anti oxidants activity .
Results in Table 3 showed the effect of flavonoids fraction isolated from ginger extract on serum MDA level and some antioxidant enzymes activity SOD, CAT in potassium oxonate hyperuricemia male rats. In hyperuricemia group there is highly significant ($p < 0.001$)increasing in MDA levels and reduction in SOD, GPx and CAT enzymes activity as compared with normal group .

Table 3: Serum Malondialdehyde and Antioxidant enzymes activities in control and hyperuricaemia rats treated with, flavoinod fraction from ginger extract after 4 weeks of treatment

Group	MDA(µM)	SOD(U/ml)	GPx(U/ml)	Cat (µM)
Normal(vehicle)	0.77±0.13	6.67±0.24	5.87±0.23	32.77±1.88
N. + F. (50µg/kg)	0.77±0.17	6.65±0.57	5.87±0.33	33.01±1.07
N. + F. (100µg/kg)	0.75±0.63	6.69±0.42	5.89±0.43	33.65±1.35
N. + F. (250µg/kg)	0.72±0.59	6.71±0.53	5.90±0.60	33.68±0.97
N. +Allopurinol	0.76±0.73	6.68±0.55	5.87±0.71	32.83±1.22
Hyperuricemia	3.66±0.48[###]	3.56±0.47[###]	3.61±0.43[###]	12.45±2.34[###]
H.+F. (50µg/kg)	2.24±0.45***	4.12±0.36***	4.11±0.37***	16.56±3.45***
H.+ F. (100µg/kg)	1.56±0.43***	4.65±0.32***	4.63±0.28***	23.78±2.90***
H.+F. (250µg/kg)	0.93±0.38***	5.56±0.29***	5.34±0.45***	29.97±2.18***
H. + Allopurinol	1.35±0.28***	4.99±0.38***	4.26±0.30***	27.35±3.16***

*** $P < 0.001$ when compared with hyperuricemia control group, [###] $P < 0.001$ when compared with vehicle control group.

  All hyperuricemia groups that treated with different concentrations flavonoids fraction isolated from ginger extract showed significant ($p < 0.001$) improvement in MDA level, SOD, CAT and GPx enzymes activity when compared with hyperuricemia untreated group, but there values showed differences according to the dose of flavonoids intake which consider that the effect of alteration of oxidative stress was dose dependent effect. The highest improvement for MDA level , SOD, GPx and CAT enzyme activities as compare with hyperuricemia

untreated group when rats intake 250 µg/kg of flavoinod fraction isolated from ginger extract. Flavonoids were very highly significant ($p < 0.001$) reduction in MDA levels (74.59%) accompanied with very highly significant ($p < 0.001$) increasing in SOD, GPx and CAT enzymes activity percent (56.18%, 47.92% and 92.28%) respectively as compared with control hyperuricemia group. Administration of flavonoids fraction from ginger extract to hyperuricemia rats after 4 weeks showed remarkably amelioration the elevation of MDA level and the reduction in SOD, GPx and CAT enzymes activity.

Discussion

Based on the results, the oral administration of flavonoids of ginger exerts notable hypouricemic effects in hyperuricemic but not in normal rats. In contrast, allopurinol reduced serum uric acid levels of both normal and hyperuricemic rats and the levels even reached to the level lower than that of normal values. These results indicate that flavoinod constituents from ginger might bring fewer side effects than allopurinol in treatment of hyperuricemia. On the other hand, this property of flavoinod constituents could be considered as an advantage for this medicinal plant. Although the elevated levels of uric acid in the circulation could give rise to gout and possibly other pathological conditions, the antioxidant action of uric acid, particularly its ability to inhibit DNA damage, is also well documented (16). Thus, excessive lowering of the uric acid level in the circulation beyond that of the normal range might even be counterproductive (16, 17). Kong *et al.* have also shown that the water extract of Ermiao wan (a Chinese herbal medicine used in the treatment of acute gout have less inhibitory effects on serum uric acid levels in normal mice compared with those animals pretreated with potassium oxonate (18).Taking into account that GF as a dietary vegetable can be used safely long-term; this feature of GF makes it a possible alternative for allopurinol, or at least in combination therapy to minimize the side-effects of allopurinol.

The results were observed flavonoids fraction of GE treated hyperuricemic rats after 14 and 28 days of intervention, serum uric acid levels reduced significantly ($p \leq 0.001$) compared to hyperuricemic control rats.Unlike these above data, the hypouricemic effect of allopurinol was statistically significant ($p \leq 0.01$) even after 1 day of the drug administration indicating the quicker onset of allopurinol action compared to that of GE, and flavonoids fraction .The inhibitory effect of GE and its major flavoinod Gingerols on serum XO activity was also confirmed in this study. XO is the key enzyme in the catabolism of purines and has a critical role in the endogenous production of uric acid (5). Several *in-vitro* studies confirmed the XO inhibitory activity of some flavonoids. These compounds are structurally similar to XO substrate and so can inhibit the enzyme activity (7). Therefore the hyperuricemic property of GE extract, observed in this study, could be explained at least in part by the inhibitory effects of them on XO activity. The extent of reduction in XO activity elicited by allopurinol was much higher than that observed when the GE administration in both normal and hyperuricemic groups.

According to these studies, the involvement of other possible mechanisms such as enhanced uric acid clearance or actions on other purine metabolizing enzymes cannot be ruled out (18, 19). This could be further supported by the existence of some hypouricemic compounds including natural products that are devoid of XO inhibitory activity (17, 18, 20). It seems that the inhibitory effects of GF, on XO activity in hyperuricemic rats are more dominant than their effects on the normal activity of either two forms of the enzyme .Similar results have been reported by Zhao *et al.* (19).

In addition, in this investigation we observed a significant increase in serum total antioxidant capacity and decrease in MDA concentration, following treatment of the hyperuricemic rats with GF. It is worth to note that GF exerts mostly their antioxidant effects in hyperuricemic groups rather than in normal groups. These phytochemicals improve total antioxidant capacity, suppress destructive oxygen free radicals and prevents oxidative stress damage (22, 23). In this study, allopurinol treatment could not significantly compensate the a based total antioxidant capacity or the elevated level of MDA concentration in hyperuricemic rats. However, the inhibition of XO by allopurinol was previously reported to decrease the level of ROS production and reduce the hepatic injury associated with liver transplantation (24).

Conclusion

Ginger flavonoids are able to reduce uric acid levels in hyperuricemic rats with no effects on the level of this biological metabolite in normal animals and prevent oxidative stress. Such hypouricemic effects may be attributed, at least in part, to XO inhibitory action of them. Therefore, the use of suboptimal dosages of allopurinol in combination with Ginger extract intake may provide a safer approach for prevention and treatment of hyperuricemia. Further investigations to explore the effect of other components of Ginger and define their clinical efficacy would be highly desirable.

References

1. Fauci A, Braunwald E, Kasper D, editors. Harrison's principle of internal medicine. New York: Mc Grow Hill Press; 2008: 2109-2115.
2. Ardan T, Kovaceva J, Cejkowa J. Comparative histochemical and immunohistochemical study on xanthine oxidoreductase/xanthine oxidase in mammalian corneal epithelium. Acta Histochem 2004; 106(1): 69-75.
3. Atlante A, Valenti D, Gagliardi S and Passarella S. A sensitive method to assay the xanthine oxidase activity in primary cultures of cerebellar granule cells. Brain Res Brain Res Protoc 2000; 6(1-2): 1-5.
4. Katzung BG, Masters SB, Trevor AJ. Basic and clinical pharmacology. 10th ed. New York: McGraw Hill Press; 2007.
5. Gonzalez, AG, Bazzocchi, IL, Moujir, L, Ravelo, AG, Correa, M.D, Gupta, MP. Xanthine oxidase inhibitory activity of some Panamanian plants from Celastraceae and Lamiaceae. *Journal of Ethnopharmacology*. 46, 1995, 25-29.
6. Kong, LD, Cai, Y, Huang, WW, Cheng, HK, Tan, RX. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *Journal of Ethnopharmacology*. 73, . 2000, 199-207.
7. Owen, PL, Johns, T. Xanthine oxidase inhibitory activity of Northeastern North American plant remedies used for gout. *Journal of Ethnopharmacology*. 64, 1999, 149-160.
8. Nijveldt, RJ, Nood, EV, Hoorn, EC, Boelens, PG, Norren, KV, Leeuwen, PAM. Flavonoids: a review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition.* 74, 2001, 418-25.
9. Shukla, Y. and Singh, M. (2007) Cancer preventive properties of ginger: a brief review, Food Chem. Toxicol., vol. 45: 683-690.
10. Jolad, S. D., Lantz, R. C., Solyom, A. M., Chen, G. J., Bates, R. B. and Timmerman,B. N. (2004) Fresh organically grown ginger (*Zingiber officinale* (composition and effects on LPS-induced PGE2 production, Phytochem., vol. 65 : 1937–1954.
11. Afshari, A. T., Alireza, S., Amirabbas, F., Ramin, S., Behrooz, Y. R. I. and Abdolamir, A. (2007) The effect of ginger on diabetic nephropathy, plasmaantioxidant capacity and lipid peroxidation in rats, Food Chem., vol. 101 :148-153.
12. Ramudu, S.K., Mllikarjuna, K. and Kesireddy, S.R. (2011a) Efficacy of ethanolic extract of ginger on kidney lipid metabolic profiles in diabetic rats, Int. J. Diabet. Dev. Ctries., vol. 31 (2): 97-103.
13. Chang, W. S., Chang, Y. H., and Chiang, H. C. (1994) Inhibitory effects of phenolics on xanthine oxidase, Anticancer Res., vol. 14: 501–506
14. Dugasani, S., Pichika, M. R., Nadarajah,V. D., Tandra, S. and Korlakunta, J. N. (2010) Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol, J.Ethnopharmac., vol. 127: 515-520.
15. Guidet, B., Shah, S. V.(1989). Am J Physiol. 257(26): 440.
16. Nuki G. Gout. *Medicine* (2006) 34: 417-423.
17. Wang Y, Zhu JX, Kong LD, Yang C, Cheng CHK and Zhang X. Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic XDH/XO activities in oxonate-treated mice. *Basic Clin. Pharmacol. Toxicol.* (2004) 94:232-237.
18. Kong LD, Yang C, Ge F, Wang HD and Song GY. A Chinese herbal medicine Ermiao wan reduces serum uric acid level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. *J. Ethnopharmacol.* (2004) 93: 325–330.
19. Zhao X, Zhu JX, Mo SF, Pan Y and Kong LD. Effects of cassia oil on serum and hepatic uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol*. (2006) 103: 357–365.
20. Zhu JX, Wang Y, Kong LD, Yang C and Zhang X. Effects of *Biota orientalis* extract an dits flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol.* (2004) 93: 133-140.
21. Yoshisue K, Masuda H, Matsushima E, Ikeda K, Nagayama S and Kawaguchi Y. Tissue distribution and biotransformation of potassium oxonate after oral administration of a novel antitumor agent (drug combination of tegafur, 5-chloro-2,4- dihydroxypyridine, and potassium oxonate) to rats. *Drug Metab. Dispos.* (2000) 28: 1162–1167.
22. . Neguyen MTT, Awale S, Tezuka Y, Shi L, Zaidi SFH, Ueda J. Hypouricemic effects of acacetin and 4, 5-*O*-dicaffeoylquinic acid methyl ester on serum uric acid levels in potassium oxonate-pretreated rats. *Biol. Pharm. Bull.* (2005) 28: 2231-2234.

23. Vijayalakshmi B and Chandrasekhar M. Oxidative stress and their resultant products as the target molecules in clinical diagnosis of age related disease. *Curr. Trends Biotechnol.Pharm.* (2008) 2: 239-250.

24. Lee WY & Lee SM. Synergistic protective effect of ischemic preconditioning and allopurinol on ischemia/reperfusion injury in rat liver. Biochem Biophys Res Commun (2006) 3: 1087–1093